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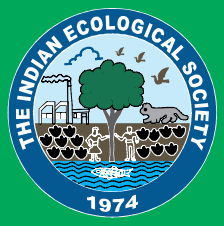

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Role of Bryophytes in Carbon Sequestration and Interactions with other Ecological Processes

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***Abstract*:** The increase in carbon levels in the environment, especially in the form of carbon dioxide (CO_2), is largely driven by human activities such as burning fossil fuels, deforestation, and industrial processes. The rise in carbon dioxide levels has a profound environmental impact, leading to global warming, higher temperatures, melting ice caps, and an increase in extreme weather events like flooding. Areas covered by bryophytes are essential in absorbing atmospheric carbon, playing a significant role in carbon cycling, particularly in sequestration and storage within ecosystems. The ability of bryophytes to sequester carbon is shaped by various factors, including environmental conditions and their biological traits. Moreover, bryophyte communities are often more responsive to environmental changes than vascular plants, suggesting possible shifts in ecosystem structure and function. This review provides a detailed examination of the role bryophytes play in carbon cycling and their potential to mitigate climate change, highlighting their importance in the global carbon cycle and their adaptability to changing environmental conditions.

***Keywords*:** Bryophytes, Carbon sequestration, Peat formation, Soil stabilization, Water retention

The increase in carbon levels in the environment, particularly in the form of carbon dioxide (CO_2), is largely driven by human activities such as fossil fuel combustion, deforestation, and industrial processes (Zukauskiene 2023, Kalashnikova et al., 2019, Sarkodie and Owusu 2016, Alsarhan et al., 2021, Ye et al., 2019). Fossil fuel combustion is a major contributor to rising atmospheric CO_2 levels (Kalashnikova et al., 2019), while deforestation disrupts the natural carbon cycle by reducing the Earth's capacity to absorb CO_2 (Zukauskiene 2023). Additionally, industrial activities, particularly those related to energy production and transportation, are key sources of CO_2 emissions (Kalashnikova et al., 2019, Sarkodie and Owusu 2016).

The environmental impacts of increasing CO_2 levels are significant, leading to global warming, rising temperatures, melting ice caps, and more frequent extreme weather events such as flooding (Zhang 2023). The connection between atmospheric CO_2 and Earth's temperature is well-documented, with CO_2 serving as a primary factor regulating the planet's temperature. Although the greenhouse effect of CO_2 has been understood for over a century, research continues to refine our understanding of its role in climate dynamics (Zukauskiene 2023). In response, various strategies, including carbon capture and utilization technologies, have been explored to mitigate the impact of CO_2 emissions (Zhou et al., 2015, Kadarukmi 2023, Li and He 2023).

Bryophytes, particularly mosses, are crucial for storing organic carbon in peatland ecosystems, playing a significant role in the terrestrial carbon cycle (Weston et al., 2014). Areas covered by bryophytes are key in absorbing atmospheric carbon, with *Sphagnum* mosses being major contributors to carbon sequestration and essential players in the global carbon cycle (Silvan and Jokinen 2016). Unlike vascular plants, bryophytes lack lignin in their cell walls, resulting in a unique structural composition (Liu et al., 2018). Due to their minimal or absent cuticle, they rely on moist environments to avoid desiccation, which also enhances their capacity to absorb atmospheric contaminants (Kosonen and Meier 2021). *Sphagnum* mosses help maintain wet, oxygen-poor, and acidic conditions that slow decomposition, leading to greater organic matter accumulation and improved carbon storage in peatlands (Sytiuk et al., 2022).

Bryophytes are crucial for capturing CO_2 through photosynthesis, reducing greenhouse gas levels, and mitigating global warming. They thrive in environments where vascular plants struggle, covering forest floors, fields, and even growing on tree trunks and rocks (Shi et al., 2021, Kasimir et al., 2021). For instance, *Polytrichum* species, or haircap moss, significantly contribute to carbon sequestration by forming organic-rich soil layers in habitats like forest floors, open fields, and tundra regions (Coxson and Marsh 2001). Similarly, *Marchantia polymorpha*, or common Liverwort, aids in soil formation and stabilization, thereby enhancing carbon sequestration. Although liverworts are generally less effective than mosses in sequestering carbon, they play a vital role in increasing soil carbon content by

colonizing disturbed or bare soils, initiating ecological succession, stabilizing soil, and boosting carbon retention in environments such as forest floors, rocks, and damp soils (Bowman et al., 2017). Furthermore, bryophytes interact with other plants and respond to environmental changes, underscoring their ecological importance for carbon sequestration and nutrient cycling (Swarnkar et al., 2024). This review explores how bryophytes sequester carbon, their role in carbon storage across various ecosystems, and their potential in mitigating climate change.

BRYOPHYTES ROLE IN CARBON SEQUESTRATION

Photosynthesis and Carbon Fixation

Bryophytes, like other plants, absorb carbon dioxide from the atmosphere through photosynthesis and convert it into organic carbon compounds essential for their growth and development. Their unique photosynthetic characteristics, such as the saturation of photosynthesis at relatively low light levels, have been well-documented in scientific literature. For example, bryophytes, including mosses and liverworts, display light-response curves indicating that photosynthesis saturates at low irradiances (Marschall and Proctor 2004). Additionally, anatomical features of bryophytes, such as their influence on non-stomatal diffusion conductance, significantly affect their photosynthetic efficiency, underscoring their adaptations to specific environmental conditions (Dangar 2024). Different bryophyte species exhibit variations in photosynthetic capacity and functional traits, with erect species allocating more nitrogen to chloroplast pigments, thereby enhancing their light-harvesting abilities compared to prostrate species (Lang et al., 2009). Bryophytes also form associations with nitrogen-fixing cyanobacteria, act as thermal insulators for the soil, and produce resistant litter. Together, these factors influence net primary productivity and heterotrophic respiration, thereby contributing to carbon fixation (Lindo et al., 2013). Their unique morphological and eco-physiological traits, such as their ability to retain moisture and withstand extreme environments, enable bryophytes to thrive in regions where most vascular plants cannot, allowing for significant carbon fixation in boreal and tropical areas (Jassey et al., 2022).

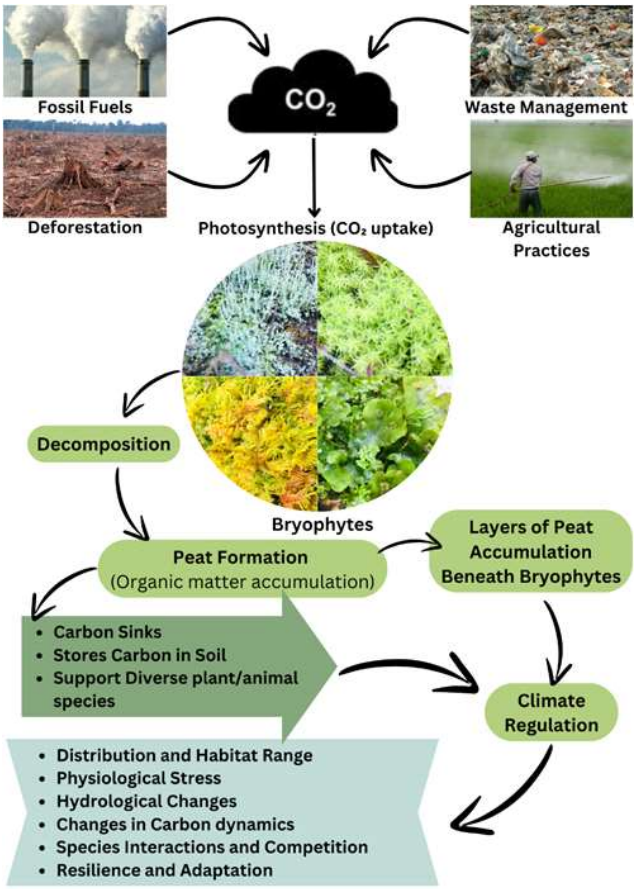

Fig. 1. Bryophyte's contribution to mitigating CO_2 emissions from various sources and climate regulation

Peat Formation

Sphagnum mosses play a critical role in the biogeochemical processes of peatlands by establishing hyperacidic, waterlogged, and anoxic conditions. These environmental factors are essential for reducing the rate of organic matter decomposition and promoting the formation of peat, a key component in long-term carbon sequestration and ecosystem regulation (Healey et al., 2023, Sytiuk et al., 2022). The accumulation of peat is facilitated by the slowed breakdown of organic material, resulting in substantial carbon storage that can persist for thousands of years. Additionally, *Sphagnum*-derived phenolic compounds contribute to this carbon storage by being more resistant to microbial degradation than the litter of vascular bog plants, thereby enhancing the longevity of carbon sequestration in peatlands (Berendse et al., 2001). *Sphagnum* mosses outcompete vascular plants and microbial decomposers by promoting their own growth while suppressing others, which reduces litter decomposition and significantly contributes to the high carbon storage capacity of peat bogs (Fudyma et al., 2019). Peatlands, which hold about one-third of the Earth's soil carbon, benefit from conditions such as high acidity, nutrient-poor environments, cold temperatures, water saturation, and anoxic conditions, all of which limit decomposition (Kostka et al., 2016). Globally, peatlands store approximately 600 gigatonnes of carbon, accumulated since the last glacial maximum, making them a persistent carbon sink throughout the Holocene (Charman et al., 2012).

BRYOPHYTES ROLE IN CARBON STORAGE

Slow Decomposition Rates

The unique chemical composition of bryophyte cell walls, particularly in *Sphagnum* mosses, significantly contributes to

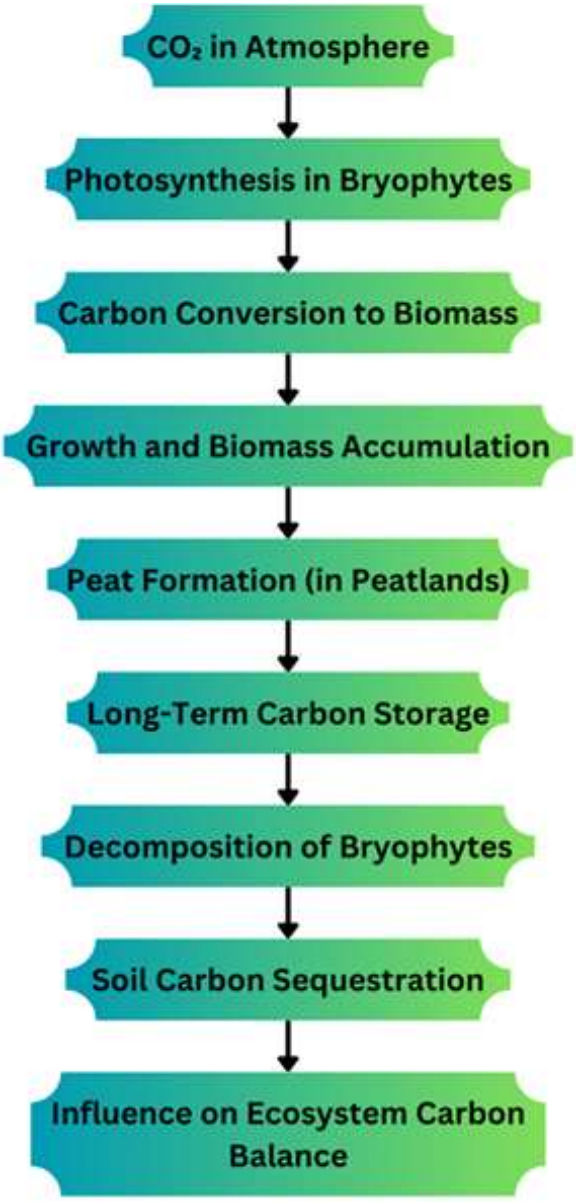

Fig. 2. Overview of the different bryophytic processes in carbon sequestration

their resistance to microbial breakdown (Hajek et al., 2010). The polysaccharides in these cell walls actively inhibit decomposition, which helps maintain the mosses' structural integrity. Additionally, high concentrations of osmolytes and the enhanced cation exchange capacity of moss cell walls increase their tolerance to environmental stressors, such as soil salinity (Lobachevska et al., 2019). In peatlands, the waterlogged conditions influence interactions between plants and microbial communities. *Sphagnum* mosses, in particular, are associated with diazotrophic methanotrophs that play essential roles in nitrogen fixation and methane oxidation, crucial processes for nutrient cycling in these ecosystems (Kox et al., 2020, Kolton et al., 2022). Peatlands are recognized as significant carbon sinks, with the rate of carbon accumulation expected to decrease gradually over millennia due to the balance between reduced net carbon accumulation in existing peatlands and the formation of new peatlands (Gorham et al., 2012).

Carbon Storage in Biomass

Bryophytes contribute significantly to carbon storage not only through peat formation but also within their own tissues (Kiebacher et al., 2023). Their unique tissue organization differentiates them from vascular plants, leading to distinct functional traits that may not align with patterns observed in vascular plant leaves or canopies (Rice et al., 2008). The poikilohydric properties of bryophytes, which allow them to retain water in varying environmental conditions, further highlight their role in ecosystem functioning and water storage. However, climate warming poses a threat to bryophytes, potentially causing severe damage to these organisms (Oishi 2018). Additionally, bryophytes are crucial for substrate revitalization, as they can accumulate carbon and mineral nutrients, supporting ecosystem health and function (Kyyak et al., 2020).

ROLE OF BRYOPHYTES IN ECOSYSTEM DYNAMICS

Bryophytes as Bioindicators in Ecosystem

The anatomical features of bryophytes make them to accumulate heavy metals such as cadmium and lead as a function of the pollution levels at both terrestrial and aquatic ecosystems (Desai et al., 2025). Bryophytes are poikilohydric organisms that quickly react on environmental variations. Altered growth, reproduction, and tissue chemistry constitute early signs of ecological stress resulting from the exposure to pollutants (Gosselin et al., 2024). Strongly linked to pollution gradients, especially in urban and industrial areas, the species distribution of bryophyte species is strongly linked to pollution gradients (Sinha et al., 2021). This aligns with the further use of some species like *Tortella tortuosa* as targeted indicators, as they are tolerant to heavy metals (Zuijlen et al., 2024). Moreover, bryophyte communities changes can indicate nutrient enrichment and eutrophication in natural waters (Fernandez-Martinez et al., 2020). It is shown in studies on *Scopelophila cataractae*, that this adaptation allows the ability of this alga to accumulate copper in the cell wall pectin, this adaptation constitutes a response to available metal levels, but also from metal stressed conditions (Sheng et al., 2023).

The metallophyte bryophytes such as *Mielichhoferia elongata* have been observed to live in metal rich soils, indicative of the broader category of the metallophyte bryophytes that inhabit adverse environmental situations. *Fontinalis* and *Brachythecium rivulare*, an indication of certain class of environmental conditions includes the presence of

iron oxides within its habitat (Glime 2024). *Polytrichum* and *Sphagnum* have also shown the extraordinary ability to bioaccumulate various mineral such as iron (Oestmann et al., 2024). For instance, *Sphagnum* mosses can change the surrounding pH to conditions promoting the precipitation of the iron minerals (Oestmann et al., 2024).

In the particular situation with forest ecosystems, bryophytes are precious indicators of microhabitat conditions which particular inestimable flora and fauna. They are good bioindicators, because of their sensitivity to environmental changes, for assessing ecosystem health and the nature of recovery to disturbances such as deforestation or climate change (Slate et al., 2024). The first is that bryophytes greatly affect the water economy or forest ecosystems. In becoming natural water reservoirs and frequently maintaining moisture levels in the forest floor they play an impact within the hydrological cycle. For example, the ability of these bryophytes to retain water, not only helps with their propagation, but also prolongs the hydration completed by the surrounding flora. In addition, epiphytic bryophytes can improve moisture retention to extend dispersion of rainfall and fog driven water to the forest floor, which may benefit seedling survival rates, promote understory biodiversity (Desai et al., 2025). This interplay illustrates the importance of bryophytes in keeping microhabitat environments normal.

Soil Stabilization and Erosion Prevention

Bryophytes, play a crucial role in stabilizing soil and preventing erosion in various ecosystems, such as forest floors, alpine regions, and along water bodies. Research indicates that bryophyte-dominated crusts enhance water infiltration, reduce runoff, and mitigate soil erosion (Seitz et al., 2017). Mosses have been shown to reduce surface runoff by up to 91% and soil erosion by nearly 100%, while increasing percolated water by 85% compared to bare soils. In temperate forests, bryophyte covers act as protective agents against soil erosion by stabilizing soil surfaces (Gall et al., 2022). Additionally, the rhizoids of bryophytes root-like structures that anchor the plants play a critical role in binding soil particles together and interweaving with adjacent plants, forming compact cushions that are resistant to detachment from the soil. This interwoven structure of bryophytes helps trap soil particles, preventing them from being carried away by water during floods (Datta et al., 2011). In particular, bryophytes such as *Barbula unguiculata* (Pottiales) and *Bryum dichotomum* (Bryales) are stress-tolerant species commonly found in areas with abundant bare soil cover. These species are typical of ground pioneer communities that are adapted to areas with strong sea aerosols and winds, characterized by their ability to colonize challenging environments (Marignani et al., 2020). Mosses like *Ceratodon purpureus* play a vital role in stabilizing extensive sand dune systems along coastlines by retaining moisture and preventing erosion. Their resilience is evident as they can survive even when covered by sand, highlighting their adaptation to harsh conditions (Moreno-Casasola 1986). In coastal dune systems, the presence of mosses such as *Ceratodon purpureus* contributes significantly to overall vegetation cover, which is crucial for stabilizing dunes and preventing their movement (Camprubi et al., 2010). Currently, mosses like *Polytrichum*, *Atrichum* and *Ceratodon* are being cultivated around fruit trees, such as apples and pears, to combat soil erosion (Groeneveld and Rochefort 2005). *Riccia* species are forming mat in terrestrial ecosystems where they apparently help to stabilize soil and control erosion as the role is still unconfirmed by any more empirical studies. This persistence under these desiccation stress conditions makes them candidate species for ecosystem restoration (Rzepczynska et al., 2022).

Water Retention and Microclimate Regulation

Bryophytes develop specialized water retention and nutrient absorption systems because their lack of vascular tissues assigns limits to their height (Rodriguez-Lopez et al., 2021). Bryophytes absorb water and nutrients directly from their environment due to their basic tissue structure. The root-like structures occurring in bryophytes called rhizoids are involved in water and nutrient absorption. Bryophytes survive in diverse environments primarily by means of capillary action, diffusion and ion exchange. The rhizoids bring about the passive movement of liquid water into the bryophyte through capillary action. The thin-walled structures maximize surface area and water contact in order to be effective absorbers. In addition to gas exchange, diffusion is important for nutrients uptake, as the minerals are diffused into rhizoid cells in concentrations gradients (Proctor and Tuba 2002). *Funaria hygrometrica* are of utmost importance for their growth under varying hydration levels, and can accommodate well to unfavorable hydration conditions only when they are in their case. Leaf shape, cell arrangement and surface modifications such as papillosity aid in water capture and minimize loss under fluctuating moisture conditions typically, unistratose leaves are the characteristic of bryophytes (Malo et al., 2025).

This water retention capability is crucial for their function and is influenced by factors such as colony structure and shoot morphology (Elumeeva et al., 2011). The water storage capacity of bryophytes varies among species and is linked to specific functional traits. For instance, deeper bryophyte layers generally exhibit a greater capacity for water storage compared to thinner layers. This enhanced water-holding capacity contributes to cooler soils and improved

temperature insulation in ecosystems where bryophytes are present (Bjorkman et al., 2019). Moss species such as *Amblystegium serpens* and *Oxyrrhynchium hians* are noted for their higher water storage capacity compared to other moss species (Thielen et al., 2021). These traits are particularly important for both bryophyte layers and biocrust bryophytes, underscoring their role in increasing water content in substrates and supporting overall ecosystem health (Slate 2024). Among the remarkable ecosystem services provided by *Riccia* species, and in particular by *Riccia fluitans*, are cyclic supply of nutrients and purification of water. *R. fluitans* is found in freshwater habitats and absorbs excess nitrogen and phosphorus to stop or limit eutrophication and improve water quality (Deilmann et al., 2024). The regulation of this nutrient supports life aquatic biodiversity by ensuring better developed aquatic habitats.

FACTORS INFLUENCING CARBON SEQUESTRATION

Species and Functional Traits

Mosses, are integral to carbon sequestration due to their unique functional traits and species-specific characteristics. Peat mosses, such as *Sphagnum* spp., are especially proficient at carbon sequestration owing to their rapid growth rates and their capacity to accumulate and preserve organic matter in peatlands (Rice et al., 2008). The functional traits of bryophytes, which are influenced by factors such as plant-soil interactions and vegetation composition, play a significant role in soil carbon sequestration amid global changes (De Deyn et al., 2008). Bryophytes form symbiotic relationships with nitrogen-fixing cyanobacteria, act as thermal insulators for the soil, and produce recalcitrant litter, all of which impact net primary productivity and heterotrophic respiration (Lindo et al., 2013). Bryophyte's presence can establish a microhabitat that allows for supporting more diverse microbial communities and interactions that thrive soil fertility. For example, bryophytes can change such processes as nitrogen fixation in soil, by their association with soil bacteria and fungi (Yang et al., 2022). Overall productivity can be improved by these microorganisms because they can improve nutrient availability to bryophytes and other surrounding vascular plants (Glime, 2024). In addition, there are some bryophytes that form symbiotic relationship with mycorrhizal fungi, so it also facilitates nutrient uptake in bryophytes and other plants around (Glime 2024). In terms of facilitating higher plant growth, bryophytes may positively influence the soil microclimate. They help regulate moisture levels, temperature, and nutrient retention through their structural features. For example, dense bryophyte cover can reduce soil erosion, maintain humidity, and mitigate temperature fluctuations, which are beneficial for the germination and establishment of vascular plant seedlings (He et al., 2016).

Although bryophytes are often categorized into functional groups due to difficulties in species identification, this approach may obscure the contributions of individual species to carbon sequestration (Lett et al., 2021). Research indicates that intraspecific variation is significant for vascular plants and lichens, while species turnover is a primary driver of trait variation in bryophytes (Zuijlen et al., 2021). Functional trait diversity is a crucial determinant of soil organic carbon sequestration across different successional trajectories (Satdichanh et al., 2023).

Water Availability

To understand the impact of drought conditions on carbon sequestration in bryophytes, it is essential to examine their reliance on water for physiological processes, growth, and photosynthesis. Bryophytes, including mosses, exhibit a range of water retention traits crucial for their adaptation and survival across different habitats (Proctor and Tuba 2002). Size, shape and aggregation of shoot morphology are of paramount importance in desiccation tolerance (DT), because of a microclimate effect granting an advantageous microclimate, which decreases the dehydration rates (Noualhaguet et al., 2023). Trapping and retaining water are one of these cushion forming colonie's pretenions to the drought or other sudden environmental changes (Elumeeva et al., 2011). Additionally, the motion of bryophytes to environmental cues, for example, leaf reorientation and shape change depending on the light intensity, allows to promote photosynthetic efficiency while minimising water loss (Kuttim et al., 2019).

Some examples of bryophyte species are mentioned below.

1. *Sphagnum palustre* are highly adapted for water retention, and what allows them to survive in saturated environments (Oestmann et al., 2024).
2. Such species as *Polytrichum commune* use their large number of rhizoids to attach to organic substratum and even to take out the moisture from ephemeral water sources to survive in different habitats (Botroh et al., 2023).
3. Thalloid form of *Marchantia polymorpha* can absorb water directly and allow gas exchange across its surface and is well adapted to moist habitats (Noualhaguet et al., 2023).
4. *Bryum argentum* form colonies can be extensive, important in trapping moisture, and have ecological functions (Deilmann et al., 2024).
5. Those species such as *Isopterygium muelleri* that grow in shady locations have larger leaves for tapping as much light as possible (Rzepczynska et al., 2022).

6. *Riccia fluitans* is known to modify its morphology in response to water levels and light intensity, and it often resides in temporary water bodies, where its survival is enabled through changes of morphology (Wang et al., 2022).
7. Furthermore, bryophytes play a role in regulating nitrogen inputs in boreal forests, which can impact forest productivity and the overall carbon sequestration response to environmental changes (Gundale et al., 2011). The water balance of bryophytes, influenced by factors such as colony structure and shoot morphology, is crucial for their habitat selection and physiological functions. This highlights the complex interplay between water availability and bryophyte ecology (Elumeeva et al., 2011).

Temperature

Temperature exerts a profound influence on bryophyte metabolism, growth rates, and the decomposition of organic matter, all of which impact their carbon sequestration capacity. Optimal temperature ranges facilitate higher growth rates and enhanced carbon sequestration in bryophytes, whereas extreme temperatures-both high and low-can stress these organisms and diminish their effectiveness in carbon sequestration (Rzepczynska et al., 2022). The temperature is a pivotal factor in shaping the carbon sequestration capacity of ecosystems. For instance, in the *Picea schrenkiana* forests of the Tianshan Mountains, China, temperature variations account for a significant portion of long-term changes in carbon sequestration capacity, with minimum temperatures playing a particularly critical role (Zhou et al., 2021). Bryophytes exhibit diverse responses to temperature fluctuations, with different species demonstrating variable growth and nitrogen fixation rates in response to temperature changes (Rzepczynska et al., 2022). Additionally, bryophytes are essential in regulating soil surface temperature and humidity, influencing organic matter decomposition, and fixing atmospheric carbon through photosynthesis, which contributes to carbon deposition in the soil. This underscores the complex relationship between temperature, bryophyte ecology, and carbon dynamics (Chen et al., 2022). Extreme temperatures, including those encountered during droughts or winter cold stress, can profoundly affect bryophyte physiology and carbon sequestration processes. During winter, when temperatures approach or fall below freezing, bryophytes experience cold stress that impairs their metabolic activities and growth (Peters et al., 2019). Temperature fluctuations can also modify soil temperature regimes, thereby influencing soil carbon and nutrient turnover. In Polar Regions, where bryophytes are pivotal in regulating soil temperature, these temperature changes can significantly impact ecosystem dynamics (Soudzilovskaia et al., 2013).

Light Availability

Light availability is a crucial determinant of the photosynthetic capacity, growth, and carbon capture of bryophytes. As photosynthetic organisms, bryophytes depend on light for energy production. In shaded environments, their photosynthetic efficiency can be limited, while areas with adequate light promote robust growth and enhanced carbon sequestration (Niinemets 2010). The availability of light is essential in shaping bryophyte communities, affecting species occurrence and abundance (Dyderski and Jagodzinski 2020). The light availability directly impacts the photosynthetic activity of bryophytes, with different species demonstrating varied responses to light intensity (Wang et al., 2015). Bryophytes exhibit specific structural adaptations to environmental conditions, including light intensity and water availability, which influence their photosynthetic capacity and growth patterns (Wang et al., 2015). Furthermore, light availability not only affects photosynthesis but also has indirect effects on moisture levels and humidity within forest ecosystems, which in turn impacts bryophyte physiology and broader ecosystem processes (Shao et al., 2023).

Nutrient Availability

Nutrient levels in soil and water have a significant impact on bryophyte growth. While some bryophytes are well-adapted to nutrient-poor environments, others benefit from moderate nutrient availability, which can enhance their growth and carbon sequestration potential. Bryophytes also play a crucial role in influencing soil nutrient availability through various mechanisms. For example, Koranda and Michelsen (2020) examined how bryophytes affect microbial decomposition processes and soil nutrient availability in a subarctic birch forest. The dead biomass of bryophytes provides to the organic matter pool and hence microbial growth and nutrient availability of surrounding plants. Hence, relatedly, there are bryophytes, including *Hylocomium splendens*, that can slow soil nutrient turnover due to their acidic litter, which alters the interactions of soil microbial communities with soil nutrients (Yang et al., 2022). Their study underscores the importance of bryophytes in regulating soil nutrient dynamics and supporting overall ecosystem function.

pH and Soil Conditions

Soils with more acidic pH levels and lower macronutrient concentrations generally support greater bryophyte coverage (Rola et al., 2021). In Hemiboreal Scots pine forests in Estonia, soil pH at the studied sites ranged from 2.1 to 3.3, reflecting highly acidic conditions. Consequently, sites

with higher pH levels tend to exhibit greater plant group richness, consistent with findings from previous research (Orumaa et al., 2022). Additionally, an increase in soil carbon, nitrogen, and organic matter content, coupled with a lower pH, has been linked to the establishment of bryophyte cover (Ortiz et al., 2023). *Hylocomium splendens*, a dominant boreal forest moss, pulls water soluble nutrients from the soil and accumulates rich, nitrate rich, organic matter of low pH. The moss's capacity to take up mineral nutrients from the environment, plus the decomposition that is derived from it, primarily contributes to soil acidity, which is this natural process more enhanced (Jaroszynska et al., 2023). More specifically, *Sphagnum* species are known for having cation exchange processes wherein proton release occurs into the soil which then decreases the pH (Oestmann et al., 2024). Soils and land managed by bryophytes have tended to accumulate organic matter in the form of soil organic carbon. The study specifically highlights that higher density of biomass of bryophyte species augments soil organic matter accumulation. In particular, bryophyte species differ in the degree to which they are capable of coping with different moisture and nutrient conditions, which improve their overall contribution to content of soil organic carbon (Rainford et al., 2022).

Disturbance and Land Use Changes

Disturbances and land use changes have a profound impact on bryophyte communities and their diversity. Research indicates that disturbances, such as treefall, can enhance bryophyte diversity in boreal forests by creating new colonization opportunities, increasing habitat heterogeneity, and facilitating the establishment of bryophyte diaspores (Jonsson and Esseen 1990). Conversely, land use changes, particularly intensified agricultural practices, can adversely affect bryophytes. For instance, Muller et al. (2012) demonstrated that fertilizer application promotes the growth of vascular plants, which compete with and suppress bryophyte populations. Fertilizers can significantly reduce bryophyte species richness, as many bryophyte species are intolerant of high nitrogen levels, which favor the growth of taller grasses and herbs and increase light competition on the forest floor. Additionally, nutrient enrichment from agricultural runoff further disrupts bryophyte habitats, posing a major threat to their communities. Hejcman et al. (2009) observed that long-term fertilizer uses results in a negative correlation between the biomass of vascular plants and bryophytes in grassland ecosystems. This suggests that increased nutrient levels favor vascular plants at the expense of bryophytes. Intensified land use can exacerbate this trend by decreasing bryophyte diversity through multiple mechanisms. These include direct mechanical impacts such as grazing and mowing, toxic effects from high nitrogen applications, and indirect effects from increased productivity that intensify competition from taller plant species (Muller et al., 2012).

Diversity and Composition of Bryophytes

The diversity and composition of bryophyte communities have a profound impact on their overall carbon sequestration capacity. Diverse bryophyte communities often exhibit complementary traits that enhance ecosystem functioning and carbon storage. The variety and composition of bryophyte species are crucial for carbon assimilation and productivity on the forest floor (DeLucia et al., 2003). Environmental factors, such as soil drainage, significantly influence bryophyte distribution and net primary productivity (NPP) (Bisbee et al., 2001). Bryophytes affect organic matter decomposition by regulating soil surface temperature and humidity, carbon fixation via photosynthesis, and carbon deposition in the soil (Chen et al., 2022).

Particularly, *Sphagnum* mosses are key ecosystem engineers in northern peatlands, where they store substantial amounts of carbon (Rice et al., 2008). These mosses form associations with nitrogen-fixing cyanobacteria, act as thermal insulators for the soil, and produce recalcitrant litter, all of which impact net primary productivity and heterotrophic respiration (Lindo et al., 2013). Additionally, the colonization of bryophytes is influenced by the diversity and composition of vascular plant species, highlighting the intricate interactions between different plant groups (Fergus et al., 2017). The factors affecting carbon sequestration in bryophytes are interconnected, involving a combination of biological interactions, environmental conditions, and land management practices.

IMPACTS OF CHANGING CLIMATE ON BRYOPHYTES

Bryophyte communities have been found to be more responsive to environmental changes than vascular plants, suggesting potential shifts in ecosystem structure and function (Post and Pedersen 2008, Oishi 2018).

Changes in Distribution and Habitat Range

As temperatures rise due to climate change, bryophytes are projected to shift their ranges towards higher elevations or poleward locations. Research shows that bryophyte diversity and distribution are influenced by elevation, temperature, and precipitation. This range shift could lead to a reduction in suitable habitats, particularly for species specialized to specific climates or microhabitats (Sun et al., 2013, Marschall 2017, Coelho et al., 2023). Warming temperatures and altered precipitation patterns present significant threats to bryophyte habitats, especially in alpine regions, boreal forests, and peatlands, where they play critical ecological roles. Studies have demonstrated that bryophyte cover and richness can decline in response to

experimental warming, with noticeable reductions observed in many species in Alpine Sweden (Sun et al., 2013).

Physiological Stress

Bryophytes, owing to their poikilohydric nature, are particularly sensitive to temperature changes because they cannot independently regulate their internal water content. This sensitivity heightens their vulnerability to desiccation and diminished photosynthetic activity under elevated temperatures. Additionally, the increase in UV-B radiation associated with climate change poses further risks to bryophytes by affecting their growth and reproduction. Due to their lack of protective cuticle layers and differentiated leaves, bryophytes are especially susceptible to UV-B radiation. Such exposure can cause DNA damage and physiological disturbances, exacerbating stress under increased UV-B conditions (Oishi 2018, Soudzilovskaia et al., 2013, Lappalainen et al., 2007). Some bryophytes, like *Bryum argenteum*, and *Marchantia polymorpha* have gone to great lengths to deal with dehydration. Gao et al. (2017) showed that desiccation tolerant bryophytes mount a successful recovery response to dehydration following transcriptional and translational controls, including the accumulation of specific proteins that stabilize cellular structures and metabolic processes during and after dehydration. Similarly, *Marchantia polymorpha* have the potential to osmoregulate during salt stress conditions where, it lacks specialized physiological mechanism halophytes and still maintain cell turgor and osmosis balance by effective water retention strategies (Lobachevska et al., 2019). It takes place by osmoregulation with accumulation of osmoprotectants such as proline, reducing osmotic potential and protection of cells. According to Ghosh et al. (2021) *Physcomitrella patens* and *Marchantia polymorpha* both respond to drought conditions with increased ABA levels correlating with increased stress resilience. For bryophytes, this adaptation is critical because the soil moisture and availability of water is highly variable.

Hydrological Changes

Changes in precipitation patterns, including increased drought frequency and alterations in snowmelt timing, can significantly impact bryophytes, which rely heavily on surface water for hydration. Reduced water availability may lead to decreased growth and potential mortality in certain bryophyte species. Despite their desiccation tolerance mechanisms, where cells can transition between full turgidity and desiccation to suspend metabolism during water scarcity (Proctor and Tuba 2002, Marschall 2017) bryophytes remain highly dependent on external water sources. Their water content diminishes rapidly with rising temperatures and decreased humidity (Oishi, 2018). Unlike vascular plants, bryophytes absorb water directly through their stems and leaves from rain, fog, or dew, highlighting their reliance on atmospheric precipitation (Coelho et al., 2023, Song et al., 2021). Peatlands, particularly in the northern hemisphere, act as crucial carbon sinks but face significant challenges due to climate change-induced factors such as warming temperatures and increased drought frequency. These conditions contribute to gradual drying and extreme weather events, which can adversely affect peatland ecosystem functions and diminish their carbon storage capacity (Kang et al., 2018, Yan et al., 2022, Koster 2023).

Changes in Carbon Dynamics

In peatlands and other ecosystems dominated by bryophytes, the impacts of climate change are deeply interconnected. Warming temperatures can accelerate decomposition processes in peatlands, potentially releasing significant amounts of stored carbon and further exacerbating climate change. As critical carbon sinks, peatlands are increasingly vulnerable to climate change-related factors such as rising temperatures and prolonged droughts, which can severely impair their capacity to function as carbon reservoirs (Larmola et al., 2014, Norby et al., 2019).

Bryophytes play a crucial role in these dynamics. In boreal forests, they help mitigate the effects of anthropogenic nitrogen inputs, which can otherwise create detrimental feedback loops in carbon cycling. Variations in bryophyte biomass influence soil temperatures, which in turn affect carbon mineralization rates and carbon sequestration in soils (Gundale et al., 2011). For instance, increased bryophyte biomass can provide insulation to the soil, thereby slowing decomposition processes and enhancing carbon storage. Moreover, the contribution of bryophytes to ecosystem CO_2 exchange is highly sensitive to rapid climate changes due to their reliance on water availability and the depth of the water table (DeLucia et al., 2003). Alterations in hydrological cycles induced by climate change can significantly impact bryophytes' ability to regulate CO_2 exchange. As ecosystems globally adapt to climate changes, the distribution of bryophyte-influenced biomes is anticipated to shift, which will, in turn, influence their contributions to global ecosystem functions (Slate 2024). The changing distribution and functionality of bryophytes across various ecosystems will be a crucial factor in future carbon dynamics and the overall health of these ecosystems.

Species Interactions and Competition

Climate change, exacerbated by factors such as pollution and habitat loss, intensifies the threats posed to bryophytes by invasive species (Lianah et al., 2021). Shifts in climate conditions can create more favorable environments for

invasive species, thereby increasing pressure on native bryophytes (Soudzilovskaia et al., 2013). Invasive species often assert dominance through mechanisms such as competition and displacement (Cheng et al., 2008). Research has shown that invasive species can negatively impact native bryophyte communities. For example, invasive species can impede the germination of native species in grassland remnants where bryophyte mats obstruct their establishment (Morgan 2006). Moreover, the spread of invasive species can alter the abundance and diversity of native bryophytes, thereby affecting overall ecosystem dynamics (Marignani et al., 2020). Species diversity of bryophytes may increase interactions within microbial community increasing substrates for microbial activity which is needed for the decomposition of organic substances and stabilization of SOC (Rainford et al., 2022).

Climate-induced changes in ecosystem structure and function can significantly influence the role of bryophytes within these systems, affecting their interactions with vascular plants, fungi, and microorganisms. These interactions are crucial for nutrient uptake and cycling. Climate changes, such as elevated CO_2 levels and warming, can impact these interactions, potentially altering nutrient availability and plant growth (Compant et al., 2010).

Resilience and Adaptation

Some bryophyte species may exhibit physiological or genetic changes in response to climate change, potentially allowing them to adapt to new environmental conditions. However, the rate and extent of these adaptations are likely to vary among species and ecosystems, reflecting the diverse ecological niches occupied by bryophytes (Slate 2024). For example, intercontinental gene flow and shared ancestral polymorphism have been identified as factors shaping the genetic structure of bryophyte populations, highlighting the complex dynamics of adaptation in these organisms (Szovenyi et al., 2008). Bryophytes, due to their sensitivity to environmental conditions, can benefit from microhabitats that offer more stable temperatures and moisture levels. Microhabitats such as tree hollows and leaf litter have been identified as crucial refuges that can help mitigate the impacts of extreme climate events on bryophyte communities (Keppel et al., 2017). These refuges provide essential conditions that support bryophyte persistence amidst changing climates

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CONCLUSION

The bryophytes play a crucial role in carbon cycling and climate change mitigation, acting as natural carbon sinks through their ability to sequester and store carbon in ecosystems. Their responsiveness to environmental changes, coupled with their widespread presence in various habitats, underscores their significance in the global carbon cycle. As climate change progresses, understanding and harnessing the carbon-sequestering potential of bryophytes will be essential for future conservation and ecosystem management strategies. Future advancements in this field could include more refined models of bryophyte carbon dynamics, increased focus on their restoration in degraded ecosystems, and deeper exploration of their interactions with other ecological processes. These advancements hold promise for enhancing our ability to mitigate climate change and sustain ecosystem health in a rapidly changing world.

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Variation in Rooting Response of Hardwood Cuttings in *Cinnamomum zeylanicum* Blume

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***Abstract*:** Efficient delivery of improved and tested germplasm in a clonally propagated tree is often influenced by tree-to-tree variation for vegetative propagation. Though cinnamon is a very important and one of the oldest known tree spices of India, individual level and seed source level variations for rooting response of stem cuttings are not very well understood. An experiment was carried out at the College of Forestry, Sirsi, UAS, Dharwad during 2019-20 in which stem cuttings from 90 morphologically superior mother trees from five different geographical sources were tested for their rooting ability in a structured experiment. Not all mother trees produced rooted cuttings. Overall, significant tree-to tree variation was observed with respect to rooting per cent (range: 20.00 to 50.00 per cent), number of shoots, number of roots and number of leaves per cutting. However, the rooting ability was not influenced by the seed-source. Per cent rooting and number of roots per cutting were positively associated suggesting that mother trees that showed good rooting abilities tended to produce larger root biomass. Differences among mother trees for rooting ability of stem cuttings is neither contributed by the age of the mother tree, nor its girth / girth of the stem cuttings used for the experimentation, pointing to the genetic influence. It is suggested to include rooting ability as an important trait while selecting the superior types in cinnamon.

***Keywords*:** Cinnamon, Vegetative propagation, Hardwood cuttings, Ortet, Rooting percent

Cinnamon, *Cinnamomum verum* Presl. (syn: *C. zeylanicum* Blume., Family: Lauraceae) is one of the earliest known and most important tree spice of India. It is cultivated as well as wild collected for its aromatic bark, leaf and immature fruits used extensively as a spice and as a medicine (Hanumantha et al., 2020; Hanumantha and Vasudeva 2022). India produces about 1659 tonnes of this spice and about 120-250 MT are imported annually. The genus *Cinnamomum* consists of about 250 species (Willis 1973) comprising of evergreen trees and shrubs. *Cinnamomum verum*, though indigenous to Sri Lanka, occurs naturally in the Western Ghats of India, which is considered as the secondary centre of origin. Cinnamon is propagated by either through seeds or clonally through cuttings or layers. Seeds of cinnamon are recalcitrant in nature and when sown immediately after harvest, they show 90-94 per cent germination; however, seeds loose viability quickly and complete loss of viability occurs after 40 days . Several workers have shown that cinnamon can be propagated through stem cuttings with three leaved young shoots (Nageswari et al., 2000, Joy et al., 2005). However, fine tune experiments to bring out the influence of seed sources and tree-to-tree variations have not been attempted (Hanumantha 2020). Such variations are important since efficient vegetative propagation and the delivery of improved and tested germplasm are at the heart of clonal horticulture or clonal forestry. These variations could be adopted in selection of higher yielding types when the traits are genetically correlated (Hanumantha and Vasudeva 2025). As propagation rates directly influence the economic viability, a challenge arises in those species which inherently have poor amenability to vegetative propagation and unpredictable rooting ability within a species.

The tree-to-tree variations for rooting ability are ignored while selecting the superior types of cinnamon perhaps because of lack good data on such variations. However in several forest tree species such variations have been thoroughly investigated and results have been mainstreamed for clonal propagation. For instance, considering over 2,39,000 stem cuttings from nearly 2200 clones of loblolly pine (*Pinus taeda* L.), Baltunis et al. (2005) have shown that rooting ability was controlled genetically with a broad sense heritability of 0.8. Studies on such variations in rooting response of cinnamon cuttings are very scanty. The present investigation was carried out to study the variation in rooting response of selected superior trees among different seed sources of *Cinnamomum zeylanicum*.

MATERIAL AND METHODS

The experiment was carried out at the College of Forestry, Sirsi, UAS, Dharwad during 2019-20 (N 14°36'21.8" and E 74°50'53.4" with temperature form 20°C to 32°C with mean annual temperature of 25 °C). Average annual rainfall of this site is 3395 mm with a mean relative humidity of 85 per cent.

During March-April 2018, about 106 morphologically superior and high yielding mother trees or candidate plus trees (CPTs) of cinnamon were selected in 8-10 years aged plantations from five different geographical sources *viz.,* Gejjehalli (Hangal), Jaddigadde (Sirsi), Kankodlu(Yellapur), Manchale (Sagar) and Siddapur (Hanumantha 2020).These sites are located right within the Western Ghats biodiversity hotspot. Cuttings were collected from 90 selected trees of cinnamon during June-July, 2019. Semi-hard wood cuttings of 20 cm length and 8.5 mm to 11.5 mm diameter with one or two leaves were prepared. The cuttings were planted in root trainers containing coir pith on the same day. Before planting, root trainers were drenched with 0.1 per cent fungicide Bavistin and finally the basal end of the stem cuttings were dipped in IBA (Indole Butyric Acid of 2000 ppm strength prepared in powder form) to enhance rooting. A total of 24 cuttings in three replications of 8 each were planted in root trainers for each selected tree and placed in a mist chamber and watering was done as when required. Planted cuttings started sprouting after 10-15 days and rooting started after one month after planting. Complete root development was noticed after 3 months. After three months the cuttings were evaluated for per cent rooting, number of shoots, number of leaves per shoot and number roots.

Statistical analysis: Data were analyzed using one way Anova in OPSTAT software.

RESULTS AND DISCUSSION

Good sprouting of stem cuttings was observed in all the 90 mother trees after two weeks of setting them in root trainers. However stem cuttings from only 26 mother trees survived beyond four weeks, suggesting an overall tree-level survival success of stem cuttings at 28.89 % (Table 1). In Siddapur source, stem cuttings from 50.00 % of mother trees showed survival followed by that of Kankodlu source (32.00%), the lowest survival success was shown in Gejjehalli source (20.00%). Overall the results indicated that out of 2160 stem cuttings set for rooting, 461 cuttings (21.34 %) showed rooting. Nageswari et al., (2000) obtained 50 per cent rooting when hard and semi-hard wood cuttings were treated with IAA at 100 ppm strength. Ananthan and Chezhiyan (2002) reported 82.6 per cent rooting of hard wood cuttings with 2500 ppm NAA. Considering the seed source as treatments, there was no significant variation in per cent rooting, mean number of shoots / roots / leaves per cutting (Table 2). This suggests that tree-to-tree variation is more important than the seed source variations. All the 26 mother trees (ortets) showed statistically significant tree-to tree variation with respect to rooting per cent, number of shoots, number of roots and number of leaves per cutting (Table 3). Ortet number S1 recorded highest rooting percent (50.00%), with highest mean number of roots (2.00), shoots (3.00) and moderately high number of leaves per cutting (4.00). The ortet number G23 showed the lowest per cent rooting (8.33 %) with relatively low number of roots/shoots (1.50) and leaves (3.83) per cutting. Co-efficient of variation was on moderately higher side in all the traits; for per cent rooting (28.97 %), roots per cutting (29.66%), number shoots

Table 1. Geo-locations, altitude, age of the plantations and sample size of mother trees considered

Seed source	District	Latitude	Longitude	Altitude (m above msl)	Age of the plantation (years)	No. of mother trees from which stem cuttings were collected	No. of mother trees showing rooting success
Gejjehalli	Haveri	N 14°44'14.9"	E 75°07'56.6"	584 m	08	25	05 (20.00 %)
Jaddigadde	Uttara Kannada	N 14°48'09.2"	E 74°44'32.9"	486 m	10	15	04 (26.6 %)
Kankodlu	Uttara Kannada	N 14°45'10.9"	E 74°50'53.9"	474 m	08	25	08 (32.00 %)
Manchale	Shimoga	N 14°10'21.9"	E 75°05'57.1"	624 m	08	15	04 (26.66%)
Siddapura	Uttara Kannada	N 14°20'14.8"	E 74°52'35.6"	584 m	9	10	05 (50.00%)
					Total	90	26 (28.89%)

Table 2. Seed source variation for percent rooting, number of shoots/roots/leaves per cutting (Mean±SEm)

Seed source	Percent rooting	Mean number of shoots per cutting	Mean number of roots per cutting	Mean number of leaves per cutting
Gejjehalli	12.50± 2.63	1.33± 0.10	2.30± 0.25	3.02± 0.29
Jaddigadde	26.04± 1.79	1.46± 0.16	1.50± 0.18	2.42± 0.20
Kankodlu	22.92± 3.16	1.42± 0.14	1.44± 0.16	3.23± 0.25
Manchale	16.67± 1.26	1.58± 0.12	1.71± 0.14	2.50± 0.24
Siddapura	25. 00± 3.22	1.61± 0.15	1.77± 0.26	2.93± 0.32
CD (p=0.05)	NS	NS	NS	NS

Table 3. Tree-to-tree variation in per cent rooting, mean number of shoots/roots/leaves per cutting in *Cinnamomum zeylanicum*

Seed source and Tree ID	Tree trunk girth (cm)	Mean diameter of stem cutting (mm)	Percent rooting of stem cuttings	Mean number of shoots per cutting after 90 days	Mean number of roots per cutting after 90 days	Mean number of leaves per cutting after 90 days
Gejjehalli						
G11	28.5	9.56	12.50a (16.90)	1.83	2.33	3.00
G16	38.0	9.20	16.67a (23.80)	1.17	1.50	2.33
G23	26.5	9.81	08.33a (13.80)	1.33	1.50	3.83
G24	39.0	10.74	12.50a (20.70)	1.00	4.33	3.83
G 25	33.0	9.17	25.00b (29.49)	1.33	1.83	2.17
CD (p=0.05)	--	0.86	(7.81)	0.30	0.74	0.85
Jaddigadde						
J1	28.0	9.01	20.83a (26.90)	1.67	1.50	2.33
J6	45.5	8.79	25.00a (30.00)	1.83	2.00	3.00
J7	43.5	9.62	25.00 a (29.49)	1.33	1.17	2.00
J25	28.2	9.12	33.33b (35.17)	1.00	1.33	2.33
CD (p=0.05)	--	NA	(5.40)	0.50	0.53	0.62
Kankodlu						
K3	33.0	9.70	25.00b (30.00)	1.17	1.33	3.33
K8	28.0	8.31	25.00b (29.49)	1.33	1.33	2.83
K9	27.5	7.70	37.50c (37.59)	2.00	1.17	4.83
K13	18.0	8.97	33.33c (35.17)	1.67	1.67	3.50
K16	23.5	9.38	25.00b (29.49)	1.83	1.17	2.83
K18	14.0	9.29	12.50a (20.70)	1.17	1.33	2.83
K20	14.0	8.94	12.50a (16.90)	1.17	1.67	3.83
K22	29.0	8.79	12.50a (20.70)	1.00	1.83	1.83
CD (p=0.05)	--	0.69	(9.15)	0.40	0.45	0.72
Manchale						
M1	18.0	9.02	16.67b (23.80)	1.50	1.67	2.67
M3	14.5	9.38	12.50a (20.70)	1.33	1.33	2.00
M7	19.0	9.12	16.67b (23.80)	1.67	1.83	2.33
M21	19.0	9.92	20.83b (26.90)	1.83	2.00	3.00
CD (p=0.05)	--	0.68	(3.820	0.36	0.42	0.73
Siddapura						
S1	39.0	8.54	50.00c (45.00)	2.00	3.00	4.00
S2	31.0	9.09	12.50a (20.70)	1.17	1.50	2.33
S5	18.0	9.27	12.50a (16.90)	1.00	1.17	2.00
S8	43.0	9.09	25.00b (30.00)	1.83	1.67	2.67
S9	50.0	11.47	25.00b (29.49)	2.00	1.50	3.67
CD (p=0.05)	--	0.95	(9.5)	0.44	0.77	0.94
Pooled over all sources						
Mean	28.80	9.27	21.31	1.47	1.72	2.90

* Values in the parenthesis indicate the arc sine transformed values
Mean values with same superscript within a seed source, do not statistically differ

(25.87%) and least for number of leaves (21.93%). Per cent rooting and number of roots per cutting were positively associated (Y = 0.064x + 0.350; R^2 = 0.855; r = 0.928) suggesting that mother trees that showed good rooting abilities tended to produce larger root biomass.

Tree to tree variation in rooting of stem cuttings reported in this study has been corroborated the results of several studies. Significant variation between cultivars and between Chemlali de Sfax clones was observed for rooting ability by Khabou and Drira (2000). Rooting of stem cuttings involve complex interactions of age of the mother tree, season of collection of stem cuttings, physiological condition of the cuttings, genetic interactions *etc*. In the current study the performance of a mother tree with respect to the per cent rooting was not significantly correlated with its trunk girth and average girth of the stem cuttings used for rooting . The age of the plantations considered in the study was similar, ranging from eight to ten years. These facts suggest that the differences among mother trees for rooting ability of stem cuttings is neither contributed by the age of the mother tree, nor its girth / girth of the stem cuttings used for the experimentation. Hence it may concluded that statistically significant tree-to-tree differences for rooting ability identified in this study perhaps represents the genetic potential of the mother trees.

Plate 1. Rooting response of hardwood cuttings in *Cinnamomum zeylanicum*

Genetic control of rooting percentages among stem cuttings has been variously attributed to provenance, family, and within-family effects. Foster et al. (2010) shown that rooting ability of stem cuttings originating from different clones(Loblolly pine) was virtually due to additive gene effects, with little evidence for dominance gene effects and with no epistasis. Baltunis et al. (2005) observed that rooting ability was controlled genetically with a broad sense heritability of 0.8 based on over 2,39,000 stem cuttings from nearly 2200 clones of loblolly pine (*Pinus taeda* L.). Shepherd et al. (2005) have shown that *P. elliottii* × *P. caribaea* hybrid families are highly variable for rooting percentage and root biomass and have moderate to strong clonal heritability. Rooting processes for the production of vegetative propagules of *Eucalyptus* such as micro-cutting and mini-cutting techniques have been used for the propagation of selected clones (superior trees), which allows considerable gains for commercial production due to higher rooting rates and a reduced time for mini-cutting formation (Stape et al., 2001, Titon et al., 2020). Chaitra Kotrabasappa Muddi and Hanumantha (2024) reported the source variation for rooting behavior in selected superior trees of cinnamon. Among the selected sources overall performance of Gejjehalli source was better (rooting: 46.67%) followed by Siddapura source with 2000 ppm IBA treatment; which may be due to family and within-family effects, genetic interactions with different concentrations of IBA treatment. The tree to tree variation for both rooting percentage and root biomass was extensive in cinnamon. The stronger genetic control and the greater economic imperative to increase rooting percentage suggest that it will have a higher priority for genetic improvement than root biomass.

CONCLUSION

Cinnamomum zeylanicum is one of the most valuable tree spices in Karnataka. Wide variability is present among the different species of cinnamon. The variations present among the different source can be used for identification of superior mother trees which respond better to vegetative reproduction. Wide variation was observed for rooting behavior among selected superior trees of different sources in *Cinnamomum zeylanicum*. Statistically significant tree-to-tree differences for rooting ability identified perhaps represents the genetic potential of the mother trees. Hence it is essential to consider the rooting ability while selecting individuals for large scale clonal propagation. The study helps to determine the variation among the selected superior trees/Candidate Plus Trees (CPT) for their rooting behavior

and selecting the superior trees in cinnamon based on their rooting ability. Superior trees with early rooting/higher per cent of rooting can be further used for mass production of elite quality seedlings and distribution to farmers.

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High-Resolution Land Use and Land Cover Mapping in Northern Region of Kashmir Himalayas Using LISS IV Data

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Abstract: Land use/cover change (LULC) studies are important for understanding environmental dynamics and ensuring sustainable management of resources. This study used LISS IV satellite data of 2016 to map and classify three forest divisions viz., Langate Forest Division (LFD), JV Forest Division (JV) and Special Forest Division Tangmarg (SFD) of Kashmir Himalayas. Using high resolution satellite data (LISS IV), the study area was delineated into 11 LULC classes: agriculture, built-up, forest, forest scrub, grassland, horticulture, snow, trees outside forest (TOF), waterbody, wetland, and wasteland. Dominant land cover was recorded in class forest with highest percentage of 49% in JV Forest division, followed by Langate forest division (42.21%) and SDF Tangmarg (22.26%). Horticulture was dominant land use in Langate Forest division (23.46%) and SDF Tangmarg (22.26%), while agriculture was most prominent in SDF Tangmarg (22.56%). Snow cover (9.26%) and wetlands (2.51%) was observed highest in SDF Tangmarg (9.26%) due to its higher altitudes. An overall accuracy of 93.52% and a kappa coefficient of 0.928 was observed. Producers and users accuracy was highest in agriculture (97.78 and 95.65%), grassland (96.43 and 96.43%) waterbody, (97.22 and 92.11%) and wasteland classes (95.45 and 95.45%). In comparison, wetland and forest classes showed slightly lower accuracies due to spectral overlap being 81.25 and 92.86% for wetland and 95.83 and 88.46% for forest. The individual and integrated LULC maps of three distinct forest divisions provided a detailed spatial representation of land use patterns, vital for decision making for land management and conservation planning. The study provide baseline data for addressing ecological challenges in the region and will contribute for future research in temporal LULC changes to assess their impact on ecosystem services and regional sustainability.

Keywords: LULC, LISS IV, Kashmir Himalaya, Forest division

Land is one of the essential natural resources, which support life and use it for a lot of developmental activities 'Hohol, Nedilska 2021).. One of the main triggers for a variety of land-based changes is land use/cover change (LUCC). LUCC affects the Earth's energy interconversion, which in turn affects the water cycle, ecosystem stability, socioeconomic aspects, and regional climate conditions (Kopittke et al., 2022, Chen et al., 2024). It provides a basis for human existence and development. LULC data is very essential for natural resource management, monitoring environmental changes, modelling, carbon cycle studies, policy formulation, hydrology, and analysis of global climate change (Song et al., 2011). Analysis of land use and land cover (LULC) provides crucial details about the region's historical development (Vijay and Varija 2024).

The LULC pattern of a region reflects the influence of natural and socioeconomic factors over time and space (Kumar et al., 2019). Unregulated changes in LULC usually degrade the environment, reduce availability of water, and lower world food security; therefore, it is a global concern. Land use is significantly affected by the interaction between natural land potential, cultural contexts, societal conditions, and physical needs (Tsai et al., 2019). Anthropogenic pressures that include population growth and increased human demand for terrestrial resources strongly alter land cover, hence causing a range of changes throughout different land systems making land a scarce resource (Shiferaw, 2011, Qasim et al., 2013, Desta and Fetene 2020, Singh et al., 2020). Hence quantifying the change in land use land cover is important for highlighting impact of human activities on the earth's surface (Deng and Quan 2023). Monitoring LULC changes gives insight into how ecosystem transformations affect the environment(Kayet, 2015, C hokkavarapu et al, 2018, Metha and Singh 2021).To address these challenges, the physical and socioeconomic drivers of land use changes and their implications in land use management policies must be understood. Despite such changes providing economic benefits, the natural environment is destabilized, affecting LULC classes and causing further ecosystem degradation (Betru et al., 2019, Schürmann et al., 2020).

Space technology developments have increased the resolution and access to remote sensing data; therefore, remote sensing data have become useful for examining local to global-scale phenomena (Rogan et al., 2008). Integrating remote sensing with GIS has improved the mapping and

classification of LULC in tremendous ways; this permits analysis on different temporal scales (Lillesand et al., 2015). Studying LULC changes requires linking a set of socioeconomic factors- economic diversification, technological advancement, population pressure among others and biophysical properties of land (Reid et al., 2000). In the Himalayan area, especially in the Kashmir Himalayas, such changes have created decreased productivity of land, therefore development concerns. Human activities in these fragile ecosystems have triggered the transformation, such as converting farmlands and abandoned areas into horticultural businesses. The main motivation is the desire to maximize profits (Shafiq et al., 2016, 2017, Fayaz et al., 2020).

The northern Kashmir Himalayas region is identified by unique topography and climatic conditions and with diverse ecosystems. The LULC in this region has registered significant changes over the recent decades. This has made changes to traditional land uses, degraded natural resources, and made it difficult to sustain in the face of development requirements. Thus, there's a need to assess these LULC patterns in this area to know the extent and intensity of change and inform some strategies to be aimed toward sustainable resource management (Wani et al., 2019, Rasool et al., 2021). .

High-resolution remote sensing data, specifically LISS-IV image, are used as valuable tools to acquire integrated information about spatial and temporal variation, especially concerning LULC phenomena (Zhu et al., 2018, Wani et al., 2019). LISS-IV sensors have fine spatial and multispectral capabilities for assessing various landscapes, especially from the Kashmir Himalayas. Once a researcher combines this data through GIS technology, then true mapping, monitoring, or classification of changes in LULC can be carried out with excellent accuracy.

The aim of this study is to identify dominant LULC classes and change over time among these divisions. The findings can help in better understanding of the dynamics involved in land use in the area and facilitate efforts toward alleviating the environmental and socio-economic consequences of LULC changes.

MATERIAL AND METHODS

Study area: This study was conducted in Langate Forest Division (LFD), JV Forest Division (JV) and Special Forest Division Tangmarg (SFD) of Kashmir Himalayas, covering a total area of 5732 km^2 (Fig. 1). The area is located at 34o0′0″ to 34o30′0″ north latitude and 74o0′0″ to 74o40′0″ east longitude and occupies northern part of Kashmir Himalayas. The area experiences temperate type of climate and with and annual rainfall of 660-1400mm and average annual temperature of around 13 °C. The study area consists of different forest types viz., lower west Himalayan temperate, dry temperate deciduous and sub alpine Fir forests, deciduous alpine scrub and alpine pastures (Wani et al., 2019). The Langate Forest Division (LFD) encompasses the regions of Mawar, Magam, Rajwar, and Rafiabad. The JV Forest Division (JV) includes the areas of Baramulla, Doabgah, Boniyar, and Uri. The Special Forest Division Tangmarg (SFD) covers Gulmarg, Soil Conservation, and Beerwah. The region is mainly composed of evergreen species including *Pinus wallichiana, Cedrus deodara, Abies pindrow and Picea smithiana* (Mehraj et al., 2025).

Land use land cover mapping: For LULC mapping, satellite data was obtained from United States Geological Survey (USGC) for 2016. The attained satellite data was pre-processed with aim for making a False Color Composite (FCC) using image processing software. Field survey was carried in study area to get primary information about the land use, topography, vegetation types and biodiversity etc. The information so generated was used in decision making while mapping. At 1:50000 scale mapping software was used in carrying out mapping of satellite data. The ground truth data collected from the field was used for accuracy assessment (Producer's Accuracy, User's Accuracy and Kappa). Different land use types (LULC) and forest classes delineated based on crown viz., Forest scrub, Grassland, Agriculture, Trees outside forest/ Agroforestry, Horticulture, Habitation, Water body, Wetland, Wasteland and Snow.

Map validation: The forest density map was generated using appropriate software and validated through ground truthing. Field data were collected for accuracy assessment, including information on forest type, latitude/longitude, altitude, crown density, tree density (per 0.1 ha), and slope percentage. An error matrix was prepared to evaluate the classification accuracy of the forest density map. Metrics such as producer's accuracy, which measures omission error and user's accuracy, which quantifies commission error, were calculated. Overall accuracy and Kappa coefficient of the map was also calculated (Congalton et al. 1983)..

RESULTS AND DISCUSSION

Eleven land classes have been identified in the study area viz., agriculture, built up land, forest, forest scrub, grassland, horticulture, snow, trees outside forest, water body, wetland, and waste land (Table 1 , Fig 2). LULC across the three forest divisions under study showed significant differences in land use pattern. Among different LULC classes, forest cover was highest in JV forest division with an area of 48,345.58 ha (49.00%) of the total area. SDF Tangmarg recorded lowest

forest cover of 16431.55 ha (18.24 %) while as Langate Forest division recorded 30,623.64 ha (42.21 %) of forest cover.

Horticulture is a major land use class in SDF Tangmarg and Langate Forest Division, covering 22.26% (20,055.36 ha) and 23.46% (17,021.87 ha), respectively, while it is significantly less in JV Forest Division (13.51%). Similarly, agricultural land use is most prominent in SDF Tangmarg, occupying 22.56%, compared to 15.34% (in Langate and only 7.78%) in JV Forest Division. Built-up land shows a higher percentage in SDF Tangmarg (8.74%) compared to JV Forest Division (6.06%) and Langate Forest Division (5.29%), reflecting greater urbanization in SDF Tangmarg. Grasslands were more prevalent in Langate Forest Division, covering 3.24% ha), while they occupy only 2.14 and 0.84% in SDF Tangmarg and JV Forest Division, respectively. Snow cover is a distinct feature of SDF Tangmarg, covering 9.26% (8,337.98 ha), indicative of its higher altitudes, whereas was minimal in JV Forest Division (0.36%) and negligible in Langate (0.05%). Wetlands were observed only in SDF Tangmarg, (2.51% -2,260.90 ha), while were absent or negligible in the other divisions.

The majority of LULC classes obtained high levels of accuracy. Among all the classes agriculture, grassland and wasteland showed both Producers and Users Accuracy exceeding 95%. In comparison wetland and forest classes recorded relatively lower accuracies. Users accuracy of 88.46% was in class forests, while as wetland class recorded Producers accuracy of 81.25%. These variations are likely due to spectral overlaps and challenges associated with distinguishing specific LULC classes. Overall accuracy of 93.52% was recorded, with an overall Kappa coefficient of 0.928 (Table 2). The maximum area under horticulture (22.26%) in the more urbanised

Some studies, in SFD Tangmarg indicate noticeable shiftfrom agriculture towards horticulture, due to the economic benefits of horticultural crops which led to rise in horticulture plantations(Shafiq et al., 2016, 2019, Mishra and Rafiq, 2017). These studies further explain that over time, a

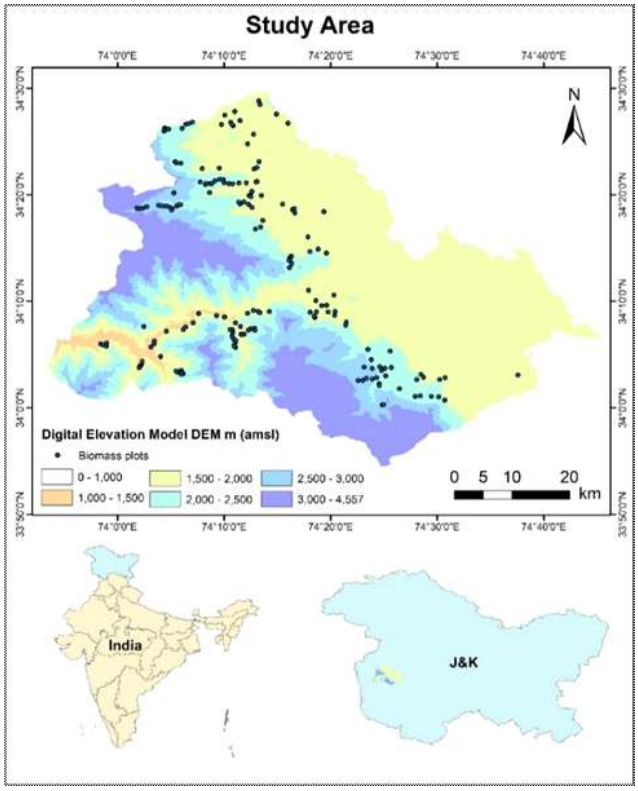

Fig. 1. Study area

Table 1. Land use land cover practices in different forest divisions

Classes	JV Forest Division		SFD Tangmarg		Langate Forest Division	
LULC	Area (ha)	%	Area (ha)	%	Area (ha)	%
Agriculture	7677.942	7.78	20325.59	22.56	11126.1	15.34
Built-up	5980.104	6.06	7877.27	8.74	3837.292	5.29
Forest	48345.58	49.00	16431.55	18.24	30623.64	42.21
Forest Scrub	3305.625	3.35	3521.66	3.91	1147.934	1.58
Grassland	829.9418	0.84	1931.04	2.14	2349.091	3.24
Horticulture	13331.46	13.51	20055.36	22.26	17021.87	23.46
Snow	355.6376	0.36	8337.98	9.26	33.2836	0.05
Trees Outside Forest (TOF)	2055.955	2.08	4928.91	5.47	2486.895	3.43
Waterbody	2066.655	2.09	2340.93	2.60	1248.537	1.72
Wetland	0	0.00	2260.90	2.51	28.95828	0.04
Wasteland	14722.22	14.92	2079.87	2.31	2639.044	3.64
Grand Total	98671.12	100	90091.05	100	72542.64	100.00

rise in temperature accompanied by a decrease in precipitation led to increasing evapotranspiration and hence adaptation of horticulture by people. Present study is in also in conformity with Fayaz et al. (2020) for the period 1992-2018 also observed that the rate of change of LULC classes was high. The area of the land use types like horticulture, sparse forest, scrub lands, pasture lands, barren lands and human settlement tends to increase. The present study revealed an overall classification of 93.52% and kappa coefficient of 0.928 for LULC classification. The LULC in south Kashmir revealed an overall accuracy of 85% and a Kappa coefficient of 0.856 for the year 2022 and the area of horticulture expanded to 1236.59 km^2, representing 22.72% of the total land area from the year 2000 to 2022(Mushtaq et al., 2024). Similarly the forest area showed an increase of 74.76 km^2 (5.05%)due to conservation efforts which is similar to present sub-study area's SFD Tangmarg, Langate Forest Division and JV Forest Division, respectively.

In all the three divisions spatially heterogeneous geographic areas are characterized by interacting patches of different diverse ecosystems, ranging from relatively natural terrestrial and aquatic systems such as pastures, forests, water bodies and plant community to human-influenced habitats and agricultural as suggested by Gardner (2015) .

The study area also has a prominence of forest cover in the form of horticulture and trees outside forests forming 15.59 % in JV forest division, 27.73% in SFD Tangmarg and 26.89% in Langate forest division. TOFs have great potential in offsetting climate change through carbon capture).Wani et al. (2019)while using high resolution LISS IV satellite data for

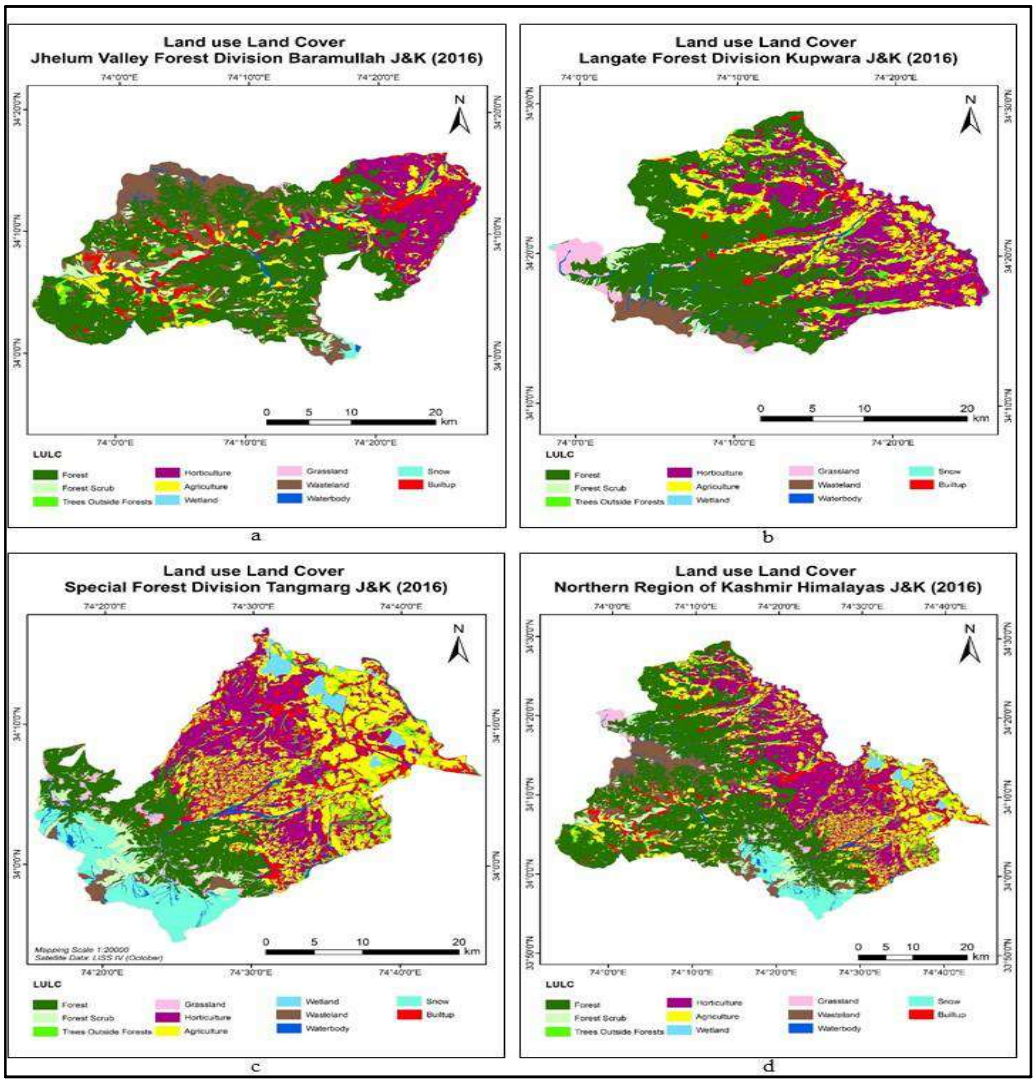

Fig. 2. LULC map of study area

assessing the area under TOF in the same study area found that the highest percentage of TOF was recorded in Special Forest Division Tangmarg that is about (5.47%) and the same was observed true for current study. Mehraj et al. (2021)using the LISS IV data for LULC classification of central region of Kashmir Himalayas observed that the overall area under TOF of district Ganderbal is 6.77% of total geographical area of the district. The overall classification accuracy of LULC map was 85.00% and kappa coefficient of 0.8333.Several other workers have carried similar studies on land use land cover classificationin different study areas (Ganguly et al., 2016, Fayaz 2023, Behera et al., 2024, Alvarez Gebelin et al., 2024, Jaiswal et al., 2025,).

The findings of this study the occurrence of different land use characteristics of each forest division. JV Forest division being predominantly natural and with minimal human intervention showed highest forest area (49.00%) among all other studied divisions. In comparison, SDF Division Tangmarg showed agriculture (22.56%) and horticulture (22.26%). Langate Forest Division exhibits a mixed landscape dominated by forests and horticulture, with noticeable grassland cover and limited snow and wetland presence. These variations underscore the importance of tailoring land management and conservation strategies to the specific ecological and socio-economic contexts of each division.

CONCLUSION

LULC mapping of the study area for 2016 identified 11 distinct land classes, indicating significant spatial variations across three forest divisions. JV forest divisions showed highest area for class forest, while SDF Tangmarg recorded the lowest forest cover In SDF Tangmarg, horticulture class represented major land use, area. Agricultural land use was most pronounced in SDF Tangmarg reflecting diverse land use practices. In case of snow and wetland classes, highest area was found in SDF Tangmarg. These findings highlight the heterogeneity in LULC patterns, driven by ecological, topographical and anthropogenic factors.

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AUTHOR's CONTRIBUTION

AAW initiated and conceptualized the study. All authors (AFB, AAW, AAG, MAI, SM and SF) contributed to field data collection and lab work. AAW and AFB contributed to data evaluation. All authors (AFB, AAW, AAG, MAI, SM and SF) contributed to writing and reviewing the manuscript.

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Comparative Anatomy for Some Species of the Genus *Lavandula* L. (Lamiaceae)

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***Abstract*:** The anatomical features of the three species of the genus *Lavandula* L. (Lamiaceae) is very important to distinguish and differentiate between *Lavandula angustifolia* Mill, *L. dentata* L and *L. stoechas* L. The results have shown that the leaf blade was uniserial epidermis in *L. angustifolia* Mill, the adaxial epidermis have small isodiametric which covered with a very thin cuticle layer, while in *L. dentate* the leaves were hypostomatic covered with a thin and striate cuticle layer, have diacytic stomata and branched non-glandular trichomes but In *L. stoechas* L. the epidermis was one layer which was rectangular cells has a thin cuticle. The glandular hairs were observed on both upper and lower epidermis.

***Keywords*:** *Lavandula*, Anatomy, Lamiaceae, Leaf blade

Lamiaceae family is the sixth largest angiosperm family contain over than 236 genera and 7173 species distributed in the world on both temperate and tropical regions (Abdel-Hady and Gamil 2018). Lamiaceae plants are generally aromatic and widely used as culinary herbs, like basil, lavender, marjoram, mint, oregano, rosemary, sage, savory and thyme (Alcione et al., 2017). Different species for Lamiaceae family contain different types, distribution, morphology, and density for glandular trichomes, that considered important taxonomic characters traditionally, taxonomy of plants based mainly on comparative morphological features that the help in taxa delimitation and identification. *Lavandula*is very important perennial shrub for the family of Labiatae (Lamiaceae), species are used for essential oils, that contain up than 300 chemical compounds. The species belongs to family Lamiaceae can be characterized using the presence of these secretion hairs. The diverse kinds of glandular and non-glandular trichomes is characteristic for Lamiaceae (Asmma and Raghad 2013). The aim of the study is to study anatomical aspects of three species of *Lavandula* for identification .

MATERIAL AND METHODS

Collection of the plants: The plant were collected from different regions of Baghdad and between 2016-2018 during the flowering period (spring). The plant were preserved in special glass ampoules containing alcohol 70% for the conducting the transverse anatomy section process.

Anatomical study: The transverse anatomy sections of stems were conducted in the using hand section (Barbara et al., 2018).

Trichomes study: The epidermis was peeled to remove the trichomes from each part of plant species for identify their typeswith the general shape and the number of cells (Durdona et al., 2020).

RESULTS AND DISCUSSION

Leaf Blade

Lavandula angustifolia Mill: The leaf blades was with uniserial epidermis, the adaxial epidermis have small isodiametric which covered with a very thin cuticle layer. The leaf abaxial epidermis have a small cells. The vascular bundles were collateral, the bundles for lateral veins have four xylem and phloem vessels. The stomata were anomocytic. So they are of different sizes. The trichomes were found in the abaxial and adaxial surfaces.

Lavandula dentate: The leaves were hypostomatic covered with a thin and striate cuticle layer, have diacytic stomata and branched non-glandular trichomes. The cells in epidermis are more sinuous from other species. The mesophyll was dorsiventral with a large vascular bundle for the midrib (Khansaa 2020). The xylem and phloem was forming the vascular sheath, and a very small amount of collenchyma, trichomes, capitate and pelleted glandular spread heavily in this species.

Lavandula stoechas: The epidermis was one layer which was rectangular cells has a thin cuticle. The glandular hairs were found on both upper and lower epidermis. Hairs branched and multicellular, 2-6 celled have smooth cuticle (Heba et al., 2013). The leaves amphistomatic have diacytic stomata. Bifacial and the palisade tissue 2 layer have spongy parenchyma 3-4 layer. Vascular bundles were collateral,

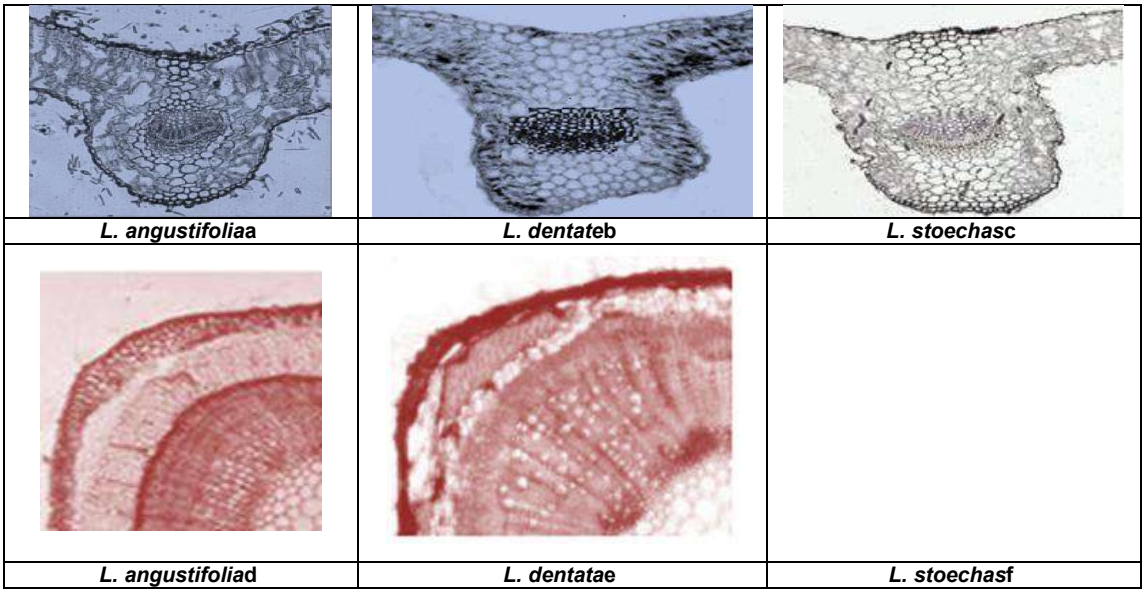

Fig. 1. Anatomical features of the three species*L. angustifolia*, *L. dentata*, *L. stoechas* a-c Anatomical sections of leaves for the three species, d-f Anatomical sections of stem for the three species

Fig. 2. Light micrographs of variety of shapes of hairs spread over the epidermis of the three species

phloem was in the abaxial, xylem was in the adaxial direction.

Stem

L. angustifolia: The stem was square shape, with angular collenchyma alternated the cortical parenchyma, so the sclerenchymatic fibers developed on the edges for the stem. It has been observed the capitate and peltate glandular trichomes with striate cuticle (Fig. 1).

L. stoechas: The stem was rectangular and the epidermis was one layer and have a thick cuticle layer. Hairs were stellate, branched. 4 rows collenchyma tissue were found at four angles, endodermis was flattened cells which distinguish from its cortex parenchyma, so the pericycle have cylindric multi-layered sclerenchyma. The complete cylinder of xylem surrounded by phloem. The pith composed of parenchymatous which covers a large area (Sevim et al., 2019).

Trichome diversity: Trichome diversity have been determined by using the light microscope. According to their morphology features (Shan et al., 2008) were divided two groups.

1. Non-glandular trichomes

-The simple non-glandular trichomes.

-The non-glandular multicellular trichomes.

-Stellate trichomes sparse stellate.

- Rough papillate.

2. Glandular trichomes multicellular

-Bifurcated glandular trichomes with different stalk lengths.

-Vesicular-capitate glandular trichomes.

-Bladde- like, head one cell, sessile, found in stem, leaves and corolla (Fig. 2). Erect-capitate glandular

Peltate glandular trichomes.

The morphological similarity alone, was insufficient criterion of delimitation for trichome types like taxonomic characters. Despite the higher number for trichome types and subtypes was known (Rajkumar 2020), their contribution for the relationships in generic level was rather small (Kim and Lee 2000). Apart from the above mentioned, the analysis was based in trichomes characters did not reveal any other trends, that would be in congruence with results obtained from different data. Glandular hairs, in the Lamiaceae, may be present at the vegetative and floral parts. In the capitula they were found at the receptacle, bracts, corolla and also on the ovary.

This paper represents the comparative analysis for leaves, stemsand trichome morphology for the three species *L. angustifolia* Mill, *L. dentata* L, *L. stoechas* L. The species contain a large variety of hairs and a dense like: non-glandular trichomes andglandular trichomes multicellular which was bifurcated glandular trichomes, Bladde- like, erect-capitate glandular and peltate glandular trichomes. The stem, leaf anatomical and trichome features provide useful characters for distinguishing the three species in the genu.

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Exploring the Nutritional Benefits and Conservation Status of Underutilized Wild Edible Plants in Rural Punjab

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***Abstract*:** Wild edible plants hold immense potential for enhancing food security, nutritional diversity, and ecological sustainability, particularly among Indigenous communities. This study evaluates the nutritional composition, medicinal relevance, and conservation status of four underutilized wild species: *Chenopodium album, Amaranthus viridis, Digera muricata,* and *Tribulus terrestris*. A meta-analysis of existing literature revealed significantly positive effect sizes *for C. album, T. terrestris*, and *A. viridis*, with overall pooled estimates of 30.63, 39.84, and 7.16, respectively. Nutritional profiling showed *A. viridis* had the highest protein (35.11%) and fibre (14.04%) content, while *T. terrestris* was richest in calcium (1600 mg/100g) and carbohydrates (50%). *C. album* emerged as a key source of iron (45 mg/100g), zinc (51 mg/100g), and magnesium (160 mg/100g). Though *A. viridis* and *T. terrestris* are listed as "Least Concern" on the IUCN Red List, C. album and D. muricata remain unevaluated, highlighting critical gaps in conservation data. Integrating these nutrient-rich species into local food systems can not only improve dietary health but also support agro-biodiversity and preserve traditional knowledge systems.

***Keywords*:** Nutrition, Diversity, Sustainable diets, Underutilized species

Indigenous peoples living in rural areas have developed profound knowledge of local biodiversity and sustainable food systems over millennia. Their traditional practices reflect a deep connection with the environment, fostering the conservation and sustainable use of natural resources, including wild edible plants. However, the encroachment of industrial agriculture, globalization and cultural disruptions have led to significant changes in their food systems. Industrialized food production and changing dietary patterns have contributed to the simplification of traditional diets, which has exacerbated nutritional deficiencies and increased health risks in these communities. These issues are further compounded by social and economic disadvantages, leaving indigenous populations more vulnerable to food insecurity and health challenges. One often overlooked aspect of indigenous food systems is the use of wild edible plants. Indigenous people living in rural areas have developed a profound understanding of biodiversity and sustainable food practices over generations. This traditional ecological knowledge is particularly valuable in the face of ongoing cultural disruptions and the global expansion of industrialized food systems. These pressures have led to the erosion of traditional diets, contributing to growing nutritional deficiencies and health problems in indigenous communities (Kuhnlein et al., 2009, Ghosh-Jerath et al., 2015).

Research indicates that *Chenopodium album* (Bathua) is rich in vitamins A and C, calcium, and iron, and possesses notable antioxidant and anti-inflammatory properties (Choudhary et al., 2020). *Amaranthus viridis* is also highly valued for its protein and micronutrient content, making it an important food source in regions affected by protein-energy malnutrition (Ganjare and Raut 2019). The decline in the utilization of these plants poses significant challenges for both nutrition and conservation. Habitat loss and the promotion of monocultures threaten the survival of these vital species. Integrating these plants back into local diets could enhance food security while promoting biodiversity conservation efforts (Subedi et al., 2006). This study examines the nutritional profiles, medicinal uses, and conservation status of five underutilized wild edible plants traditionally consumed by indigenous communities: *Chenopodium album, Amaranthus viridis, Digera muricata, and Tribulus terrestris*. These plants are often overlooked in modern food systems despite their significant health and ecological value.

MATERIAL AND METHODS

An extensive literature survey was conducted through the Web of Science database (http://apps.webofknowledge .com/), Google Scholar (http://scholar.google.com). To retrieve articles from the databases, the following search terms were used: "*Chenopodium album*" / "*Tribulus terrestris*" / "*Amaranthus viridis*" / "*Digera muricata*" OR "nutrient" / "medicinal" / "macronutrient" / "micronutrient" / "vitamins" / "nutritional profile" / "mineral composition/ "N" / "P" / "K" / "Ca" / "Mg" / "S" / "NPK"/ "Zn" / "Fe" / "protein" / "Calcium" / "Sulphur. To find pertinent information, references to connected articles were also examined. Peer-reviewed

journal articles published from 2000and few articles from 1900 were shortlisted following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2017 and Fig. 1). We screened the retrieved articles and retained those that met the following four inclusion criteria: (i)The study contains nutritional profile of plant (ii) The study excluded other quality attributes such appearance, textural qualities, aroma, dimensions, hue, splitting, and illness incidence in favor of concentrating on quality criteria that were associated with the mass concentration of nutritionally significant elements. Software called JASP was used to analyze the data and standard error. Responses that did not overlap with zero in the 95% CI were considered significant ($p < 0.05$).

RESULTS AND DISCUSSION

Overview of analysed data: The forest plot illustrates the effect sizes and corresponding confidence intervals for individual studies included in the meta-analysis. Most studies investigating *Chenopodium album* in Figure 2 reported positive effect sizes, indicating a generally beneficial impact of the treatment or intervention. Notably, studies such as Poonia and Upadhayay (2015), Yadav and Sehgal (1999), and Yadav and Sehgal (1999.2) demonstrated large and statistically significant effect sizes, as their confidence intervals do not cross the zero-effect line. However, Pandey and Gupta (2014), reported non-significant results as their confidence intervals crossed the zero-effect line, suggesting uncertainty regarding the true effect. The overall effect size, represented by the diamond shape at the bottom of the plot, was estimated to be 30.63, with a confidence interval ranging from -2.19 to 63.44, as calculated using a random-effects model. While this suggests a generally positive effect, the wide confidence interval reflects a high degree of variability among the included studies, potentially indicating heterogeneity in study design, interventions, or outcomes. This variability should be explored further to better understand its impact on the overall findings.

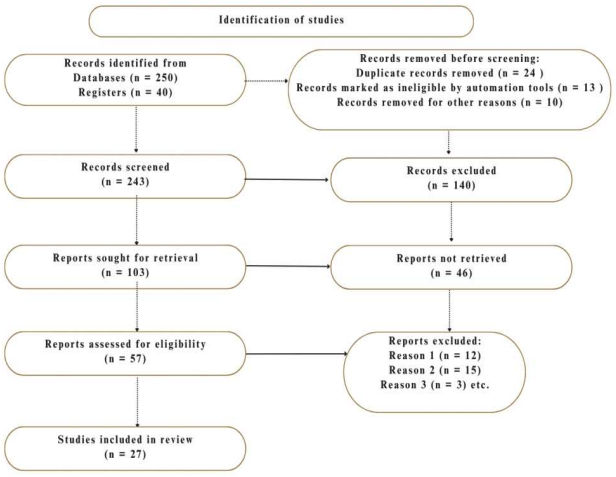

Fig. 1. Schematic diagram of identifying studies for analysis

The forest plots presented in Figures 3, 4, and 5 summarize the effect sizes and 95% confidence intervals for studies evaluating the impact of *Tribulus terrestris, Digera muricata, and Amaranthus viridis*, respectively. In Figure 3, most studies on *Tribulus terrestris* report positive effect sizes, suggesting a generally beneficial impact of the intervention. Khalid et al. (2023) and Saeed et al. (2024) report strong and statistically significant results, with effect sizes of 21.33 [20.74, 21.92] and 220.00 [198.87, 241.13], respectively. These narrow confidence intervals that do not cross zero indicate high precision and reliability. However, studies such as Tkachenko et al. (2020) report an effect size of 0.00 [–0.20, 0.20], while 'Semerdjieva et al. (2019.4) shows very wide confidence intervals, indicating non-significant or highly uncertain results. The pooled effect size using a random-effects model was 39.84 [–2.19, 81.88], suggesting a positive trend with substantial heterogeneity. In Figure 4, which evaluates D. muricata, most studies also report positive outcomes. Verma et al. (2016) and Saeed et al. (2024) demonstrate strong effects with values of 24.10 [23.98, 24.22] and 220.00 [198.87, 241.13], respectively. Conversely, Gupta et al. (2005) and Saeed et al. (2024) report non-significant findings, The pooled effect size for Digera muricata was 50.69 [6.19, 95.19], indicating a significant overall effect. Lastly, Figure 5 shows the effect sizes for Amaranthus viridis. Studies such as Gupta et al. (2005.1), Umar et al. (2011), and Xarvier et al. (2018) report statistically significant and precise outcomes with effect sizes of 35.11 [32.76, 37.46], 21.05 [19.33, 22.77], and 24.54 [24.13, 24.95], respectively. However, several studies including Sarker and Oba (2019), and Achigan-Dako et al. (2014) show minimal or non-significant effects with confidence intervals that cross zero. The pooled estimate from the random-effects model for *Amaranthus viridis* was 7.16 [1.08, 13.25], indicating a statistically significant, though more modest, overall effect.

Nutritional Profile of Plants: Based on the combined data (Figure 2, 3, 4, 5), *Chenopodium album* exhibited a moderate fat content of 2.5% and a protein level of 12%. Its carbohydrate content was 25%, and it also contained 5% crude fibre. Mineral analysis revealed substantial amounts of potassium (400 mg/100g), magnesium (160 mg/100g), and calcium (150 mg/100g). Additionally, C. album was rich in iron (45 mg/100g) and manganese (1.2 mg/100g), indicating its potential to contribute to dietary micronutrient intake, particularly in relation to oxygen transport and antioxidant

defense. *Tribulus terrestris* showed a high carbohydrate content of 50% and a protein level of 10%, making it a valuable source of plant-based energy and protein. Although its fat content was relatively low (1.1%), it contained significant levels of calcium (1600 mg/100g), magnesium (130 mg/100g), and potassium (300 mg/100g), all essential for bone development, muscle function, and cardiovascular health. Iron content was also notable at 9.2 mg/100g, along with trace amounts of zinc and copper. *Digera arvensis* presented a balanced nutritional profile, with 15% crude fibre, 8.75% protein, 48% carbohydrates, and 1.5% fat. It was moderately rich in minerals such as calcium (145 mg/100g), potassium (350 mg/100g), and magnesium (120 mg/100g). Although its iron content (6 mg/100g) and copper (0.5 mg/100g) were lower compared to the other species, it still contributes to the intake of essential micronutrients. *Amaranthus viridis* stood out as the most nutrient-dense species, with the highest recorded protein content (35.11%) and crude fibre (14.04%). It also contained 0.47% fat and 7.67% carbohydrates. Mineral analysis showed good levels of magnesium (100 mg/100g), potassium (310 mg/100g), and calcium (140 mg/100g), along with iron (20 mg/100g) and manganese (1.0 mg/100g). These values highlight its potential as a functional food with significant nutritional benefits, especially for addressing protein and micronutrient deficiencies.

Recent assessments from globally recognized conservation databases such as the IUCN Red List and

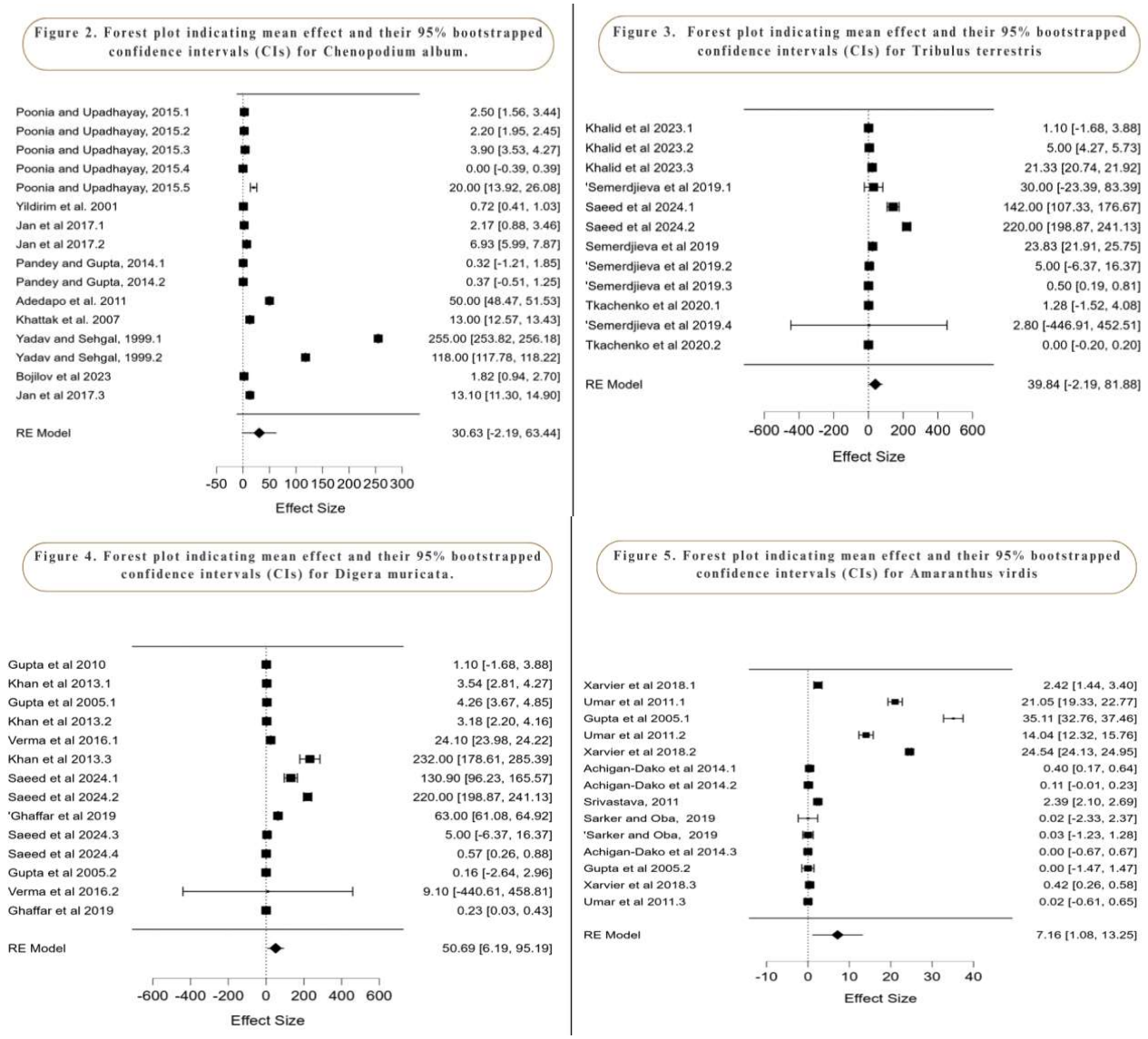

Fig. 2-5. Forest plot indicating mean effect and their 95% bootstrapped confidence intervals (CIs) for *Chenopodium album, Tribulus terrestris, Digera muricata and Amaranthus virdis*

Table 1. Nutritional composition of four wild edible plants (per 100 g dry weight), showing macronutrient and micronutrient values in *Chenopodium album, Tribulus terrestris, Digera arvensis,* and *Amaranthus viridis*

Plant name / Nutrient dose	*Chenopodium album*	*Tribulus terrestris*	*Digera arvensis*	*Amaranthus viridis*
Crude fibre (%)	5	1.3	15	1.93
Fat (%)	2.5	1.1	1.5	0.47
Carbohydrate (%)	25	50	48	7.67
Protein (%)	12	10	8.75	2.11
Ash (%)	4	4.6	8	1.85
Zn (mg)	51	1	2	2
Mg (mg)	160	130	120	100
P (mg)	80	70	90	85
K (mg)	400	300	350	310
Na (mg)	10	15	12	11
Ca (mg)	150	1600	145	140
Cu (mg)	0.8	0.6	0.5	0.4
Mn (mg)	1.2	1.1	1.3	1
Fe (mg)	45	9.2	6	20

Plants of the World Online (POWO) indicate that *Tribulus terrestris* and *Amaranthus viridis* are currently classified as species of “Least Concern.” This status reflects their wide distribution, ecological adaptability, and stable population trends, suggesting that they are not facing immediate threats to their survival. In contrast, the conservation status of *Chenopodium album* and *Digera arvensis* (syn. *Digera muricata*) remains largely undocumented or unassessed by major international conservation authorities. These species are not currently evaluated in the IUCN Red List, and their population data are sparse in databases such as the Global Biodiversity Information Facility (GBIF). Despite their traditional use and nutritional value, limited ecological studies have been conducted on their habitat dynamics, population trends, or potential threats. This knowledge gap highlights the urgent need for targeted conservation research to determine their vulnerability, especially in regions where natural habitats are increasingly being modified by agricultural intensification and urban expansion. Integrating these lesser-known wild edibles into conservation planning and biodiversity monitoring could help ensure their long-term sustainability, while also preserving traditional ecological knowledge associated with their use.

The nutritional and medicinal relevance of underutilized wild edible plants is increasingly recognized in sustainable food system research. This study evaluated *Chenopodium album*, *Amaranthus viridis*, *Digera arvensis*, and *Tribulus terrestris*, confirming their potential in addressing nutritional deficiencies and preserving traditional food knowledge. *Chenopodium album* is known for its high levels of micronutrients such as calcium, iron, and zinc, along with antioxidants and phenolics. Singh et al. (2023) documented its antimicrobial and anti-inflammatory properties, affirming its nutritional and therapeutic relevance. *Amaranthus viridis* displayed the highest protein and fibre content among the studied plants. Silva et al. (2021) highlighted its phenolic and flavonoid bioaccessibility and strong antioxidant activity, supporting its role as a functional food. Although *Digera arvensis* is less researched, Gupta et al. (2010) demonstrated its good nutritional potential and traditional usage in rural diets. Its incorporation into community-based nutrition strategies could support food security efforts, especially in undernourished populations. *Tribulus terrestris* stood out for its exceptionally high calcium content and saponin-rich phytochemistry. Saeed et al. (2024) emphasized its pharmacological roles, including anti-inflammatory and potential anti-obesity effects, making it a multipurpose nutraceutical candidate. While *A. viridis* and *T. terrestris* are listed as “Least Concern” by the IUCN, the absence of conservation data for *C. album* and *D. arvensis* warrants ecological assessments. Promoting the cultivation and integration of these species into local food systems can address malnutrition, enhance biodiversity, and preserve indigenous agricultural practices

CONCLUSION

This study highlights the nutritional significance, medicinal potential, and conservation relevance of five underutilized wild edible plants traditionally consumed by Indigenous communities: *Chenopodium album*, *Amaranthus viridis*, *Digera muricata*, and *Tribulus terrestris*. Meta-analysis and nutritional profiling revealed that these species

are rich in essential macro- and micronutrients, particularly protein, iron, calcium, and dietary fibre—making them valuable contributors to food and nutritional security. Among them, *Amaranthus viridis* and *Tribulus terrestris* demonstrated the highest protein and mineral contents, while *Chenopodium album* showed considerable micronutrient density. Despite their traditional importance, *Chenopodium album* and *Digera muricata* remain underrepresented in global conservation assessments, emphasizing the need for further ecological monitoring. Reintegration of these plants into contemporary diets and farming systems could address micronutrient deficiencies, support sustainable food systems, and conserve traditional ecological knowledge. Overall, this study advocates for the recognition and revitalization of wild edible plants as viable components of climate-resilient and culturally respectful food strategies.

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Assessment of Root-Shoot Growth of Tree Seedlings Raised in Conventional Container Type and Air-prune Pots

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Abstract: The experiment investigated the effects of different growing environments on various shoot and root characteristics of six tree species, including *Terminalia bellirica*, *Tecomella undulata*, *Terminalia arjuna*, *Azadirachta indica, Syzygium cumini*, and, *Madhuca longifolia* over a period twelve months. The seedlings of 30 days growth were transplanted into four container environments: black polybags kept on cemented floor (C1), white polybags kept on nursery floor (C2), black polybags kept on nursery floor (C3), and airpots (C4), using a factorial completely randomized design with four replications. There were significant differences for various traits except root-shoot ratio. After one year of growth, *Terminalia arjuna* continued to show superior growth in height (67.36 cm) while *Terminalia bellerica* for collar diameter (8.62 mm). However, the reverse trend was observed for tap root length and diameter. The seedling fresh (79.65 g/seedling) and dry (33.37 g/seedling) biomass was highest for *Syzygium cumini*. *Maduca longifolia* produced the lowest seedling growth and biomass except tap root diameter in *Tecomella undulata*. The container environment (C3) showed the maximum values for root shoot growth and biomass production due to higher production of lateral roots. Quality assessments indicated that the good root shoot ratio and sturdiness quotient (SQ) were obtained for *Syzygium cumini* in air-prune pots; and *Terminalia bellerica* in air-prune pots. The maximum Dickson's quality index (DQI) was observed for S5C3 followed by S4C3 and S5C2. Besides, root shoot ratio and SQ, DQI was significantly maximum for the seedling grown in black polybags kept on nursery floor.

Keywords: Forest tree seedling, Container type, Air-prune pots, Root-shoot growth

Forest cover plays a pivotal role in maintaining ecological balance, particularly in a biodiversity-rich country like India. According to the Forest Survey of India (FSI 2021), the total forest cover in India spans 713,789 square kilometres, accounting for 21.71% of the country's geographical area. Additionally, trees outside forests contribute another 95,748 square kilometres, or 2.91%, resulting in a combined forest and tree cover of 24.62%. Despite this, the forest and tree cover in some regions, such as Punjab, remains critically low. In Punjab, forest and tree cover constitutes only 3.67 and 2.26%, respectively, of the state's total geographical area, which is very low by 33% minimum forest cover recommended by the National Forest Policy (1988) for well being of human life and ecological sustainability. This alarmingly low forest cover in Punjab has sparked significant ecological concerns, prompting a series of afforestation efforts led by the state forest department, non-governmental organizations (NGOs), and local communities.

Despite these efforts, environmentalists and NGOs have raised concerns about the poor survival rates of out-planted saplings, attributing the issue to inadequate nursery practices that result in low-quality seedlings. Surveys of forest department nurseries revealed that 30-40% of tree stock, aged 1 to 3 years, exhibited severe root deformities, including multiple or rudimentary taproots, fewer fibrous roots, and girdled roots. Saplings also demonstrated irregular stem thickness and leaning central leaders, with destructive analysis revealing coiled and kinked roots, which could impede long-term establishment. These deformities are a major concern, as a well-developed root system is essential for sapling survival and successful establishment in the field (Grossnickle 2005).

Poor root quality significantly hinders a tree's ability to adapt post-transplantation, underscoring the need for improved nursery management practices to ensure successful reforestation and afforestation efforts. A healthy root system is not only vital for nutrient and water uptake but also serves as a foundation for sapling sturdiness and growth. Previous studies have shown that factors such as species selection, the rooting environment, and post-planting care play critical roles in seedling survival and establishment (Hirons and Percival 2012). Furthermore, advancements in nursery practices, particularly the shift toward container nurseries in the 1970s, have allowed for greater control over seedling production by enabling nurseries to optimize cultural practices (Dumroese et al 2016). These containerized systems, however, have introduced new challenges, particularly in root development, as the confined growing space can lead to issues such as root circling, girdling, and kinking, which compromise the sapling's ability

to establish itself in the field (Gilman and Kempf 2009). Container design, growing medium composition, and root pruning are crucial factors that influence the development of healthy seedlings in nurseries. Research indicates that containers designed to encourage the formation of fibrous root systems are more effective at promoting nutrient uptake and overall tree establishment (Arnold and McDonald, 2006). Root pruning, a key nursery practice, fosters a more robust lateral root system, enhancing the sapling's ability to absorb nutrients and water post-transplantation (Gilman and Wiese 2012). However, improper pruning techniques can lead to undesirable root deformities, which may negatively impact long-term growth and survival in unmanaged site conditions. This study assessed the seedlings of different forest tree species grown in different containers including nursery polythene bags air-prune pots for root-shoot growth, seedling biomass characteristics and quality of seedlings after 12 months of transplanting.

MATERIAL AND METHODS

Experimental site and plant material: The study was carried out at Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana, Punjab, India (30°54' N latitude and 75°48' E longitude, 247 m amsl). Twelve-month-old tree seedlings of *Terminalia bellirica*, *Tecomella undulata*, *Azadirachta indica*, *Terminalia arjuna*, *Syzygium cumini* and *Madhuca longifolia* were used for study. The nursery seedlings were bare-rooted and were subjected to corrective pruning to remove any root defects prior to planting in the containers. The potting media comprised volumetric proportions of soil:vermicompost (3:1; v/v). The pots were tapped 3-4 times while filling of the media to ensure uniform bulk density throughout its finite volume.

Cultural practices: The one-month-old seedlings of uniform size (4-5 inch length) were planted in four containers, *i.e.* black polybags kept on hard floor (C1), white polybags kept on nursery floor (C2), black polybags kept on nursery floor (C3) and air-prune pots (C4). The polythene bags are having size of 8×6 inches and experiment was in the factorial completely randomized design with 4 replications with a plot size of 5 seedlings per treatment. The experiment was initiated on August 2022. The pots were filled with loam soil : sand : farm yard manure in equivalent volumetric ratios by gentle tapping while filling the media mixture to ensure uniform bulk density and similar compaction levels. The rooted seedlings of uniform age (one-month-old) were transplanted into polythene bags and air-prune pots. The pots were kept under 50% shade net ensuring only 50% of the sunlight light transmittance during first week of May till end of June. During rest of the year, the pots were kept under open sun ensuring uniform sunlight and gaseous exchange with the surrounding air. The pots were irrigated as and when required in August-September, and thereafter the frequency of irrigation was reduced once per week (October-February) due to low temperature. The pots were irrigated daily with the rise in temperature (March-July) ensuring thorough wetting of the pot volume at nearly 100% saturation. The weed growth in pots was controlled by periodic hand weeding. The seedlings were uniformly supplemented with recommend doses of fertilizers.

Growth and biomass measurements: The various observations were recorded during September 2023 on 12-months after transplanting of seedling in the pots to monitor the growth rate. Shoot growth was assessed in terms of seedling height, number of leaves and branches, fresh weight and dry weight of the shoots. The collar diameter was measured with a digital vernier caliper gently placed 1 cm above the collar region. The observations pertaining to root characteristics were categorised as root length and diameter of main tap root and number of first-order lateral roots (FOLR, with diameter ~1-2 mm) and second order lateral roots (SOLR, with diameter < 1mm). Further, fresh and dry biomass of the root system was also calculated to assess the variability in dry matter accumulated due to type of pots. The characteristics defining the quality of seedlings were also assessed by determining the root-to-shoot ratio, sturdiness quotient (SQ), and Dickson's quality index (DQI).

Statistical analysis: The statistical analysis was carried out using SPSS software version 21.

RESULTS AND DISCUSSION

The tree species and container types significantly affected seedling growth and biomass, but no significant effect was observed for the ratio of root-to-shoot biomass (Table 1). The container size had larger effects on seedling growth and biomass production of seedlings except root weight than genotype of seedlings. There was significant interaction between tree species and container types for all the trait of the seedlings used in the study.

Shoot characteristics: The seedling height ranged from 31.3 cm (*A. indica* grown in C4) to 83.08 cm (*T. arjuna* in C3), while the root-collar diameter varied from 4.64 mm (*S. cumini* in C4) to 10.19 mm (*T. arjuna* in C3) as presented in Table 2. Number of leaves and branches varied from 11.75 (*T. arjuna* in C4) to 68.67 (*T. bellerica* in C3), and from 1.33 (*S. cumnini* in C4) to 4.33 (*T. arjuna* in C3), respectively. These findings illustrate the pronounced impact of various growing containers on the height development of the tree seedlings, highlighting the importance of selecting appropriate cultivation conditions for optimal growth. The study

order of S6C4 > S3C4 > S5C4 > S1C1 > S2C4 >S1C3 > S5C1. The mean maximum mean root-to-shoot ratio was obtained for air-prune pots (0.84) and *S. cumini* (0.72). A small sturdiness quotient (SQ) indicates a sturdy seedling with higher chance of survival under field conditions. For those air-prune pots seedlings, *i.e. M. longifolia* (S6C4; 4.03), *A. indica* (S3C4; 4.52), *S. cumini* (S5C1; 5.11) and *T. bellerica* (S1C1; 5.59) seedlings showed the good SQ values (SQ < 6.0). Remaining seedlings grown in different container type are somewhat susceptible to some environmental or cultural damages due to having higher SQ, *i.e.* >6.0 value, thought their growth was higher in terms of shoot or root growth. Dickson's quality index (DQI) is one of the important parameter used to assess the seedling vigour at minimum 60 days of age. However, in the present study, the seedling were evaluated at the age of 12 month after transplanting, the maximum DQI was observed for S5C3 (4.54) followed by S4C3 (4.05) and S5C2 (3.72). Besides root shoot ratio, DQI

Table 2. Effect of genotype and container type on shoot growth in forest tree seedlings

Species	Container	Seedling height (cm)	Collar diameter (mm)	Number of leaves	Number of branches
Terminalia bellirica (S1)	C1	51.29	8.05	41.08	1.83
	C2	55.57	8.66	52.39	2.53
	C3	62.42	9.11	68.67	2.17
	C4	60.75	8.66	60.83	1.58
	Mean	57.51	8.62	55.74	2.03
Tecomella undulata (S2)	C1	61.00	8.47	21.25	1.50
	C2	57.43	6.61	23.50	3.17
	C3	61.94	9.65	41.56	3.26
	C4	45.94	7.14	30.36	2.56
	Mean	56.58	7.97	29.17	2.62
Azadirachta indica (S3)	C1	66.71	6.41	28.83	1.50
	C2	62.25	6.32	29.33	2.42
	C3	71.08	8.07	33.11	2.47
	C4	31.11	6.04	14.67	1.58
	Mean	57.79	6.71	26.49	1.99
Terminalia arjuna (S4)	C1	71.56	7.88	22.25	2.67
	C2	70.72	9.15	30.92	3.39
	C3	83.08	10.19	44.92	4.33
	C4	44.08	6.72	11.75	1.75
	Mean	67.36	8.49	27.46	3.04
Syzygium cumini (S5)	C1	45.61	8.10	51.19	3.58
	C2	60.87	8.19	42.94	2.44
	C3	62.47	8.77	53.00	3.69
	C4	40.39	4.64	21.78	1.33
	Mean	52.34	7.43	42.23	2.76
Madhuca longifolia (S6)	C1	58.67	5.59	30.47	3.22
	C2	57.92	6.65	25.75	2.08
	C3	59.31	7.28	33.25	3.83
	C4	28.44	5.00	12.67	1.61
	Mean	51.09	6.13	25.54	2.69
CD (p=0.05) Tree species		4.48	1.01	3.92	1.01
Container type		5.17	1.23	4.70	1.23
Species × Container		7.67	1.47	7.41	2.47

confirmed that all species and growing containers differed significantly in terms of shoot growth. Seedlings raised in black polybags placed on the nursery floor showed the highest values, suggesting that the container and environment significantly impacted growth. These findings align with earlier studies on the impact of container types and growing environments on seedling growth. Venkatesh et al. (2002) reported that *Acacia nilotica* seedlings grown in large size black polythene bags exhibited more shoot length, leaf count and collar circumference. Similarly, Malik and Shamet (2009), Ferdousee et al. (2010) observed that the large size and bottom-hole polybags, significantly influenced seedling growth metrics in nurseries. Oliet et al. (2005) and Grossnickle and South (2014) confirmed that container size significantly affects the early growth and root morphology of tree species. Grossnickle and South (2014) discussed the significance of container design and size directly affects the root morphology and overall seedling vigor, supporting the claims made also by Malik and Shamet (2009).

Root characteristics: The effect of the genotype and container type on root characters of seedling was significant (Table 3). The root length and diameter varied from 29.33 cm (*A. indica* in C2) to 47.25 cm (*T. bellerica* in C3), and from 5.52 mm (*S. cumini* in C4) to 11.31 mm (*T. arjuna* in C3), respectively. In term of number of first order and second order lateral roots were significantly affected with the container type and varied from 6.08 (*T. bellerica* in C4) to 28.17 (*S. cumini* in C4), and from 10.61 (*T. undulata* in C4) to 52.92 (*S. cumini* in C3), respectively. The seedling of *S. cumini* showed lower diameter with higher number of first and second order laterals roots. Root length and diameter showed opposite trend with shoot length and diameter. The colour of the containers has significant difference on seedlings root growth. Approximately 20-30% more root length and number of lateral roots in black polybags kept in nursery floor than white polybags kept in nursery floor except *S. cumini* and *M. longifolia*; however, root diameter does not have any significant effect of colour of container. Similar to these results, best seedling growth and morphology of *Hevea brasiliensis* roots was observed in larger containers (Salisu et al., 2018). Amal and Mohamed (2010) explored that large and container and black colour had better root development and better growth in *Eucalyptus camaldulensis* seedlings. Vieira et al. (2019) concluded that shoot and root growth of *Agave angustifolia* seedlings had significant growth differences due to container size, shape, and growing media composition.

Biomass characteristics: The fresh and dry biomass of shoot and root in forest tree seedlings grown in the containers C3 were highest than C1, C2 and C4 (Table 4). Fresh shoot and root biomass varied from 21.05 g/seedling (*A. indica* grown in C4) to 59.52 g/seedling (*S. cumini* grown in C3), and from 8.64 g/seedling (*M. longifolia* in C4) to 33.41 g/seedling (*S. cumini* in C3), respectively. The minimum dry shoot and root weight was observed in *M. longifolia* seedling grown in C4 (5.38 and 3.01 g/seedling). However, the maximum dry shoot and root weight was 26.43 g/seedling for *T. undulata* and 13.83 g/seedling for *A. indica* grown in C3 containers, respectively. *Terminalia bellerica*, *T. undulata* and *A. indica* did not show any significant differences. The attributes of the biomass varied significantly among the different tree species, while, no significant differences were observed for root fresh and dry weigh. Growing container significantly affects seedling biomass. In contrast, there were no discernible variations in dry weight of the roots and shoots when seedlings grown in transparent and black polybags (Nayanakantha et al., 2018). Tsakaldimi et al. (2005) studied the effect of container type on the root growth and morphology of *Quercus ilex* and *Pinus pinea*, and concluded that deeper and larger containers promoted greater root depth, better anchorage, and increased survival rates in the field, mirroring the observations of Nayanakantha et al. (2018) regarding the importance of container size and type.

Seedling quality characteristics: Owing to the interaction between tree species and container type, we evaluated the integrated effect of treatments on the seedling quality characteristics, *i.e.* root-to-shoot ratio (Fig. 1), sturdiness quotient (Fig. 2) and Dickson's quality index (Fig. 3). Significant differences were observed for tree species, container and their interactions (Fig. 1-3). The root-to-shoot biomass ratios ranged from 0.47 to 1.03, and followed the

Table 1. Analysis of variance for growth and biomass production of forest tree seedlings

Factors	Seedling growth				Seedling fresh biomass		
	Height (cm)	Collar diameter (mm)	Root length (cm)	Root diameter (mm)	Shoot weight (g)	Root weight (g)	Root-to-shoot ratio
Species (S)	10.67***	4.61***	4.12**	3.80**	2.56***	2.59***	0.94 ns
Container (C)	14.63**	6.88**	5.11***	7.30***	6.76**	1.53***	1.54 ns
S×C	9.92***	3.19**	2.63***	2.48***	4.34***	1.64*	0.78*

Note: ns: non-significant. *, ** and *** indicate significant effects at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

was significantly maximum (3.33) in C3 container type.

The present study supports the assertion of Singh *et al* (2024) that tree seedlings grown in air-pruning containers and found that air-pruning improved root architecture, resulting in better root-to-shoot ratios and increased survival rates in field conditions. Sharma et al. (2017) observed the use of air-pruning containers on the growth of *E. camaldulensis* seedlings and concluded that seedlings grown in air-pots had superior root systems, including increased lateral root development and reduced root circling. This enhanced root structure promoted better shoot growth and increased plant stability post-transplantation. The seedling grown in air-pruning containers had a well-structured and well developed fibrous root system (Mariotti et al., 2015). Chiatante et al. (2015) explored the use of air-pruning containers in enhancing the growth of *Populus alba* and *Pinus halepensis* seedlings and findings showed that air-pruning containers produced seedlings with more

Table 3. Effect of genotype and container type on root growth in forest tree seedlings

Species		Container	Root length	Root diameter	FOLR	SOLR
Terminalia bellirica (S1)		C1	43.56	9.18	10.81	35.67
		C2	35.22	9.16	12.58	29.44
		C3	47.25	9.73	16.83	43.25
		C4	37.41	8.46	6.08	24.25
		Mean	40.86	9.13	11.83	33.15
Tecomella undulata (S2)		C1	30.08	6.77	11.33	19.00
		C2	32.33	6.75	12.17	10.61
		C3	42.00	7.54	18.31	25.53
		C4	35.87	5.59	12.28	23.75
		Mean	35.07	6.66	13.52	19.72
Azadirachta indica (S3)		C1	31.83	7.45	7.10	22.00
		C2	29.33	8.18	9.67	21.21
		C3	40.00	8.95	11.25	20.17
		C4	30.43	6.89	10.22	22.67
		Mean	32.90	7.87	9.56	21.51
Terminalia arjuna (S4)		C1	36.31	9.85	17.31	50.67
		C2	37.00	10.50	27.72	46.33
		C3	42.00	11.31	22.25	61.25
		C4	31.48	7.01	21.67	28.67
		Mean	36.70	9.67	22.24	46.73
Syzygium cumini (S5)		C1	33.58	8.93	22.78	36.97
		C2	37.88	8.82	26.17	50.92
		C3	38.46	9.38	28.17	52.92
		C4	36.41	5.52	22.00	38.39
		Mean	36.58	8.16	24.78	44.80
Madhuca longifolia (S6)		C1	30.04	7.15	6.83	23.81
		C2	32.22	7.05	6.64	25.42
		C3	32.78	8.56	10.58	23.25
		C4	29.42	7.05	7.11	16.11
		Mean	31.12	7.45	7.79	22.15
CD (p=0.05)	Tree species		3.22	1.05	1.99	9.26
	Container type		3.61	1.29	2.44	11.34
	Species × Container		6.23	2.58	2.88	12.54

Note: FOLR - First order lateral root; **SOLR** - Second order lateral root

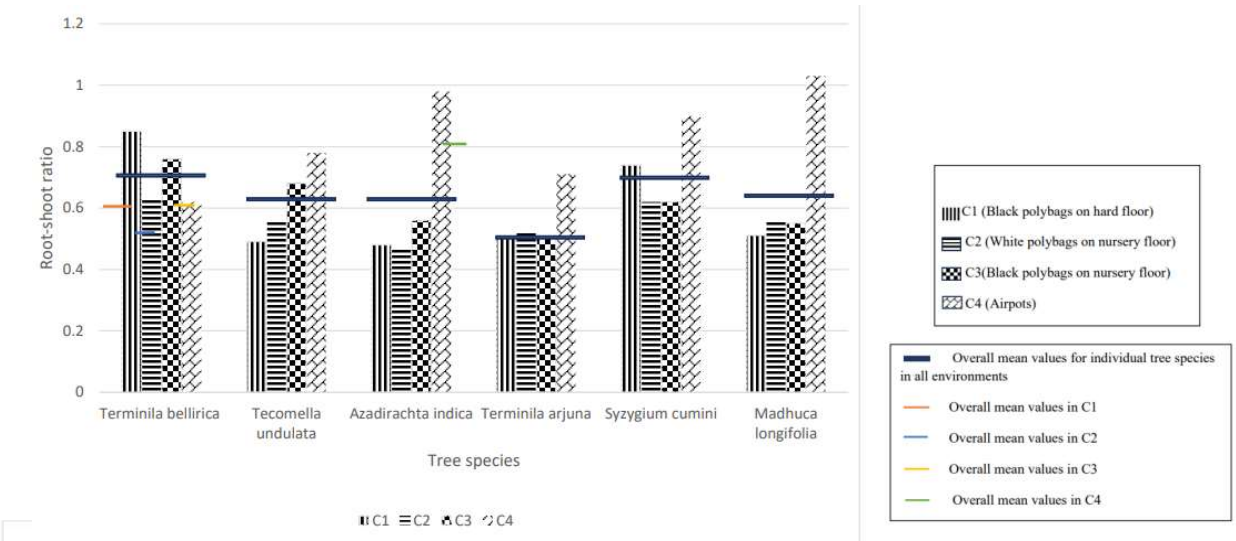

Fig. 1. Effect of container type on root-shoot ratio in forest tree seedlings

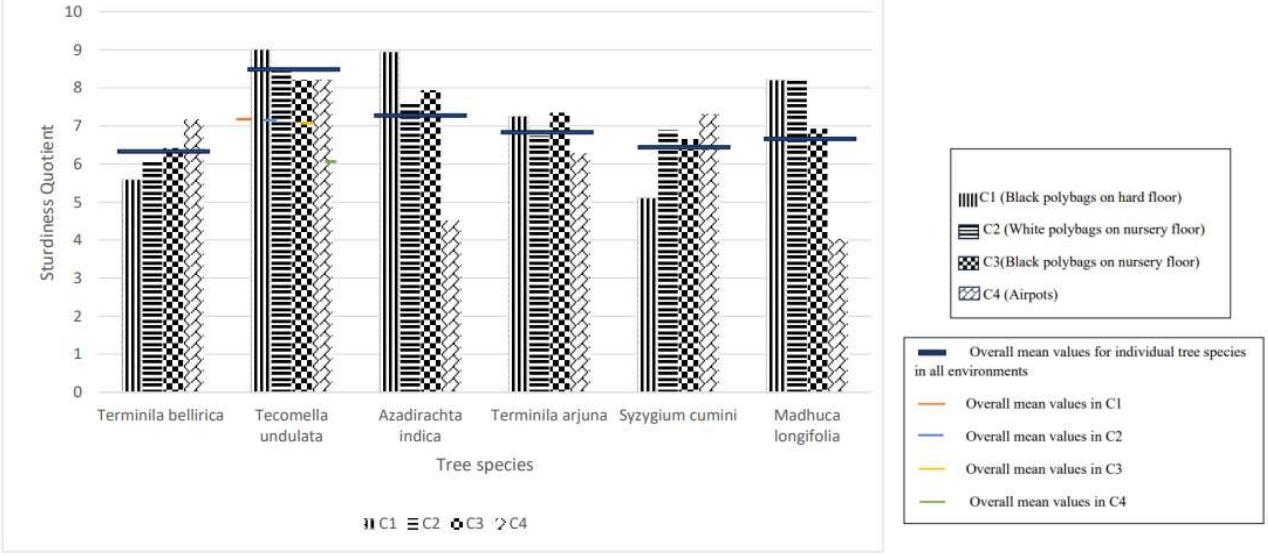

Fig. 2. Effect of container type on sturdiness quotient in forest tree seedlings

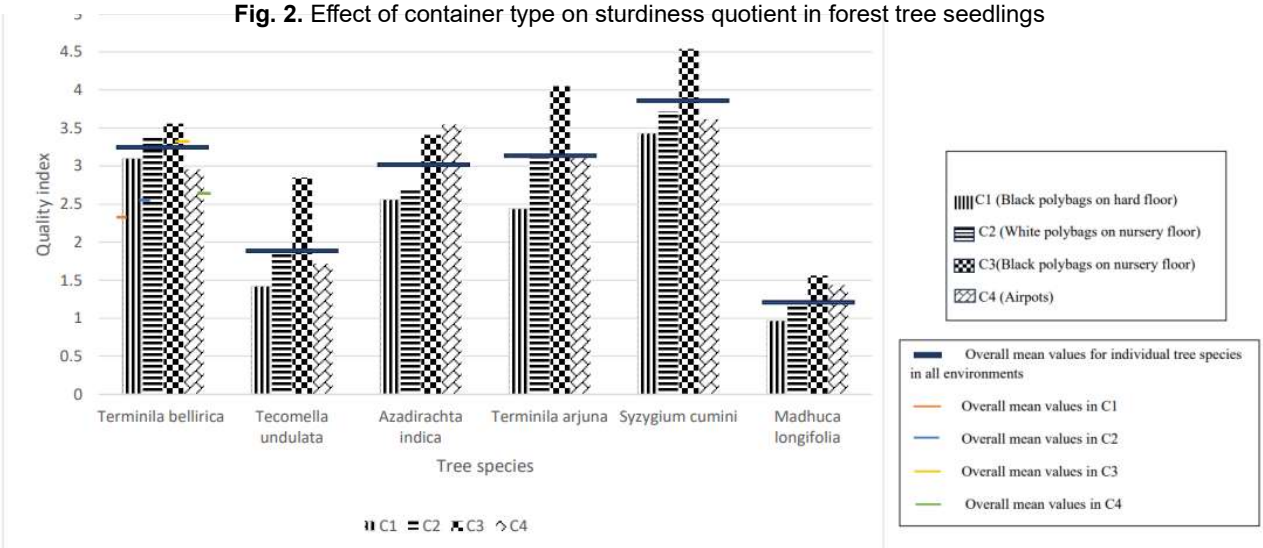

Fig. 3. Response of tree species on Dickson's quality index in forest tree seedlings

Table 4. Effect of genotype and container type on biomass production in forest tree seedlings

Species	Container	Fresh weight		Dry weight	
		Shoot	Root	Shoot	Root
Terminalia bellirica (S1)	C1	26.28	23.67	11.62	9.47
	C2	31.88	27.42	13.87	10.98
	C3	34.82	32.23	17.45	11.00
	C4	32.04	28.99	14.26	10.89
	Mean	31.26	28.08	14.30	10.59
Tecomella undulata (S2)	C1	43.60	14.01	11.97	4.53
	C2	44.59	16.73	15.36	5.73
	C3	56.97	20.09	26.43	7.30
	C4	27.15	14.82	9.51	6.99
	Mean	43.08	16.41	15.82	6.14
Azadirachta indica (S3)	C1	32.15	28.17	12.35	12.98
	C2	28.53	29.39	11.83	11.37
	C3	36.98	33.14	17.58	13.83
	C4	21.05	23.25	9.28	10.04
	Mean	29.68	28.49	12.76	12.06
Terminalia arjuna (S4)	C1	41.73	19.56	16.33	7.03
	C2	42.30	20.46	17.51	9.33
	C3	53.43	25.46	24.83	12.77
	C4	48.52	18.42	21.58	7.31
	Mean	46.50	20.98	20.06	9.11
Syzygium cumini (S5)	C1	48.46	18.07	22.62	6.61
	C2	52.84	25.35	22.39	10.89
	C3	59.52	33.41	26.22	13.08
	C4	50.79	30.16	18.77	12.88
	Mean	52.90	26.75	22.50	10.87
Madhuca longifolia (S6)	C1	26.16	10.36	7.29	3.03
	C2	28.18	10.95	6.80	4.54
	C3	35.15	15.61	9.46	4.60
	C4	22.98	8.64	5.38	3.01
	Mean	28.12	11.39	7.23	3.80
CD (p=0.05) Tree species		7.44	NS	5.82	NS
Container type		11.01	7.35	6.81	4.73
Species × Container		18.03	9.71	10.62	NS

extensive and deeper root systems, increased root branching, and better overall plant health. The study emphasized that air-pruning prevented root deformities such as root circling, contributing to better transplant success. These studies further confirm the benefits of air-pruning containers, such as air-pots, on seedling root and shoot development. By promoting a well-structured and fibrous root system, air-pruning containers improve water and nutrient uptake, reduce root deformation (e.g., circling), and lead to better post-planting performance.

CONCLUSIONS

The study highlights the significant influence of container environments on root-shoot growth, biomass production, and seedling quality in six forest tree species. Seedling quality depends not only on height and diameter but also on attributes like leaf count, mini branches, and lateral roots. Superior growth was observed in *T. bellerica*, *T. arjuna*, and *S. cumini*, recommended for afforestation and roadside plantations. Black polybags (C3 container type) provided optimal conditions for growth, while air-prune pots (C4

container type) improved root-to-shoot ratios and sturdiness quotient (SQ). The study underscores the importance of container environment and species selection in nursery practices and need to explore for further research on long-term field performances of these tree saplings.

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In Vitro Studies on Gametangial Ontogeny and Development of Gametophyte of Homosporous Fern- *Dryopteris chrysocoma*

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***Abstract*:** In the present study spores of *Dryopteris chrysocoma* were cultured in P & T medium and 67.47% and spore germination was recorded within a week of sowing. Spore germination was found to be Vittaria Type. The viability of spores was lost in ten months, when kept in room temperature (20-25°C). Prothallial development was Aspidium Type. In vitro culture creates a new path for mass cultivation of ferns and will be very helpful for their restoration and conservation. This work advances our knowledge of the pteriodophytes reproductive capacity, enabling us to better preserve, restore, grow, and protect them.

Keywords: *Dryopteris chrysocoma*, Gametophyte development, *in vitro,* Mass propagation

In addition to having tremendous decorative potential, pteridophytes have demonstrated their abilities as phytoremediators, bio-indicators for pollution, and bio-fertilizers. They are a type of ecological indicator and typically develop in areas where flowering plants may not thrive. India is home to 12000 living species of ferns and about 1000 species of fern allies. Fern vegetation is threatened by anthropogenic influences, natural disasters, unplanned urbanization, industrialization, agricultural extension, and soil erosion and due to these large populations of terrestrial and epiphytic ferns, as well as other angiosperms are now extinct.

Dryopteris chrysocoma is a member of the dryopteridaceae family, which has 1600 species and 45 genera, making it one of the largest leptosporangiate groups. This species is distinguished by a short, ascending, thick, and tufted rhizome. It flourishes in temperate parts of Europe, the British Isles, and Asia. *Dryopteris chrysocoma*is prevalent in 2,000-3,000m altitude range near Darjeeling in India. According to reports, the chemical components of *Dryopteris chrysocoma* include albaspidin, filmaron, oleoresin, flavaspidic acid, and filicic acid (Alam 2010). Root of *Dryopteris chrysocoma* has anthelmintic properties and is mainly used to eliminate tap worms. Ferns are used as medicine by several cultures, including the Reangs and Chorai and recently several ferns and fern relatives have been used for various medical treatments, biofertilizers, and as nursery plants. They can also be employed as hyper accumulators of toxic metals and as pollution indicators (Rani 2022).

The *in vitro* culture approach opens up a new avenue for fern conservation to meet the need for plant resources for commercial and restoration efforts. The morphology of the gametophyte dramatically changes as it develop, modifying tissue architecture for newly differentiated cells. Growth of the gametophyte in ferns follows a developmental progression to gather more and more photosynthates in order to get ready for the reproductive phase. Fern gametophyte develops quickly in the culture medium, and the effect of culture medium, growth regulators, and culture conditions easily affect spore germination and gametophyte development. Gametophytes are nutritionally independent, which makes it easier to conduct experiments, make observations, and expose objects to light. The mass production of ferns will benefit greatly from *in vitro* spore culture. The production of a large population of gametophytes from spore germination in tissue culture allows us to monitor developmental patterns and investigate the role of growth regulators. The fern gametophyte is a perfect model system for the study of physiology, photobiology, and cell biology (Fernandez 2003). The tissue culture method of spore germination enables the production of spore populations free from contamination by spores of other species, infection by bacteria and fungi, and interaction with algae and mosses, all of which are common problems when developing in the natural environment.

MATERIAL AND METHODS

Specimen collection: In plastic bags, mature fertile fronds of *Dryopteris chrysocoma* from Mussoorie, Uttarakhand, were collected. The fronds were kept at room temperature in brown spore packets in desiccators with silica gel.

Spore inoculation and sterilization: For two minutes, the spores were maintained in a solution of 2% sodium hypochlorite in water. Parker's macronutrient culture medium and Thompson's micronutrient culture medium were used as the culture medium for spore sowing.

Scanning electron microscope studies: Electron microscopy was used to study spore morphology. Spores were dried and adhered on the stubs using sticky tape before being scanned at various magnifications in a sputter coater (JFC-1600 Auto Coater, JEOL Japan).

RESULTS AND DISCUSSION

Spore Germination

Dryopteris chrysocoma produced pale brown, bilateral, and perinate spores. The ornamentation on the exine and perine (or either of them) is frequently made of granulose or spinulose (Fig. 1 A, B) and measure 27.13 x 33.04µm. After 7–10 days of inoculation, spore germination was observed 67.47%. Spore germination was Vittaria type and the spore coat was observed to separate at the laesura area. The chlorophyllous protonemal cell and first rhizoid were followed by a series of cell divisions (Fig. 1, C). The spores have a conspicuous loose perine that has wrinkled subannular or elongated folds. Because of their limited spore viability and other physiological factors, ferns need more time to generate sporophytes than other angiosperms. The taxonomy of fern species has an impact on spore germination. A successfully fertilized spore forms a gametophyte, which then develops into a sporophyte.

Prothallus Development

After 15 to 18 days of sowing, two-dimensional protonemal cells were visible, followed by the spatulate stage after 21 to 26 days (Fig. 1, E), and cordate gametophytes after 35 to 38 days (Fig. 1, F). The protothallial development was Aspidium type. The mature prothallus was huge, cordate, and thalloid, with thick midribs that were typically nearly as wide as the wings. The gametophyte had a small apical cleft in its anterior area and numerous brown-colored rhizoids and was symmetrical in nature. The adult prothallus has unicellular secretory papillate hairs all around and is heavily haired. The protonemal starting cell split into five to six cells, which generated a filamentous protonema (Fig. 1, D). Spore viability vanished completely at room temperature after 200 days. *Dryopteris chrysocoma* spores that had germinated produced filamentous prothalli that eventually transformed into cordate gametophytes. The spores produced filamentous prothalli, which eventually transformed into heart-shaped gametophytes. The gametophyte of *Dryopteris chrysocoma* is symmetrical in nature, has a shallow apical notch, and has numerous brown colored rhizoids on its posterior area. The adult prothallus of this species is cordate, thalloid, and large, with thick midribs that are typically almost as wide spread-out as the wings. Each spore in the study produced one gametophyte, which developed from a filamentous stage to a two-dimensional heart-shaped stage.

Gametangial Development

Dryopteris chrysocoma has typical Leptosporangiate-type sex organs. Following maturation, the cordate gametophyte remained in a vegetative state for almost 70 days until gametangia began to develop. After 90–95 days of spore germination, the posterior area between the rhizoids on the ventral surface of the thallus began to develop antheridia (Fig. 1, I). After 100–115 days of spore germination, archegonia began to develop on the ventral surface of the thallus, right below the apical notch along the anterior portion of the midrib (Fig. 1, F). The gametophyte develops bisexuality within four months, reaching its peak after 110 days of spore seeding. Table 2 appends the specific sex ontogeny events. Antheridia in the current study developed earlier than archegonia on the same prothallus by at least 10 days to ensure cross-fertilization and genetic diversity in nature. Sporophytes in *Dryopteris chrysocoma* did not begin to form until 180 days after spore sowing. A morphological investigation of gametophytes that were unable to create sporophytes at the end of the experiment revealed that they had functioning archegonia but had depleted antheridia. A small number of functional antheridia were also found, which may have been caused by antheridiogen involvement. The absence of sporophyte production can also be explained by different combinations of gametophytic lethal (Wu 2021).

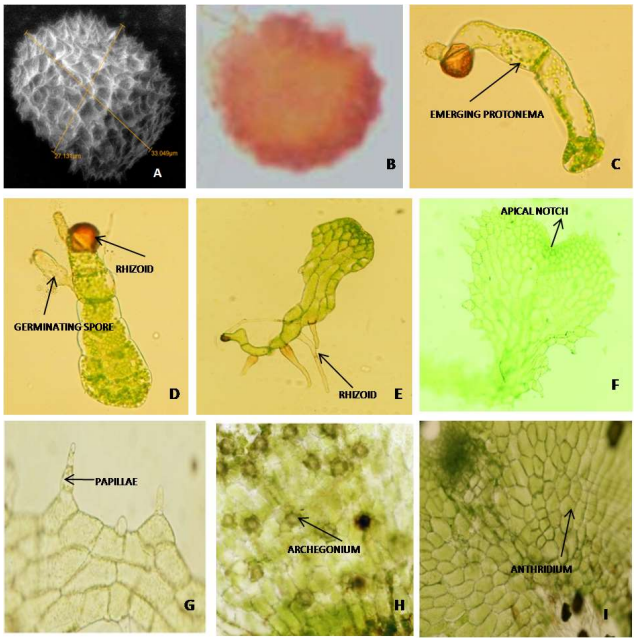

Fig. 1. *Dryopteris chrysocoma*: A, B: Spores, C: Emerging protonema, D: Filamentous stage, E: Spatulate stage, F: Cordate gametophyte, G: Close view of Marginal unicellular hair H: Archegonia below apical notch, I: Antheridia in between rhizoid

Table 1. Event of spore germination and gametophyte development of *Dryopteris chrysocoma.*

Day after sowing	Spore germination (%)	Number of protonemal cell	Number of rhizoidoidal cells	Two dimensional stage (%)	Spatulate stage (%)	Cordate stage (%)
7	67.47±1.87	1±0.03	0.6±0.58	0	0	0
14	73.96±2.32	3±1.03	1.0±0.55	0	0	0
21	80.85±1.06	7±0.6	1.1±0.4	18±0.9	0	0
28	84.35±1.43	10±0.4	1.3±0.43	36±0.75	0	0
35	96.36±0.69	19±2.08	4.2±0.77	68±0.4	8±0.63	2±0.02
42	100	59±1.38	15.1±0.8	76±0.39	36±0.25	8±0.4
49	100	105±0.6	26.0±0.56	100	78±0.19	10±0.23
56	100	156±0.41	37.7±0.35	100	100	30±0.67
63	100	169±0.32	48.2±0.69	100	100	80±0.96
70	100	192±0.61	53.4±0.41	100	100	100

Parentheses showing Mean± Standard Deviation

Table 2. Chronological changes in sex ratio in a composite culture of *Dryopteris chrysocoma.*

Day after sowing	Number of neuter	Number of male	Number of female	Number of bisexual
80	20±0.3	0	0	0
90	14±0.69	1±0.63	0	0
100	11±0.21	4±0.89	1±0.36	0
110	6±0.69	5±0.23	1±0.21	1±0.42
120	7±0.84	10±0.48	2±0.64	1±0.13
130	1±0.66	11±0.32	3±0.98	2±0.28
140	0	11±0.14	5±0.78	4±0.14
150	2±0.87	3±0.19	6±0.16	3±038
160	3±0.1	4±0.73	10±0.35	6±0.43

According to studies (Bharati 2013, Parihar 2010) low germination rates, fungal and algal contamination, sporophyte development, and growth inhibition brought on by invading species can all be overcome through in vitro culture. Rare species like *Angiopterisboivinii* (Seychelles) and *Cibotiumschiedei* (Central America) are observed to demonstrate good growth through in vitro culture (Goswami 2016). By adjusting nutrients like nitrogen and carbon in the medium, one can accomplish the early creation of the gametangia. The gametophyte needs almost three months in the regular nutritional medium to create gametangia (Suneetha 2022). The maturity of spores at the time of collecting may have an impact on different growth patterns. The variable responses of various fern species may also be explained by the spore viability at the moment of collection.

The gametophyte not only permits sexual reproduction but also affects migration, recruitment, habitat choice, and adaption (Li 2022). Little research has been done on important aspects of gametophyte biology, such as the selection of habitats by gametophytes based on their morphological and physiological diversity, the timing and method of development and maturation, the breeding system and habitats that produce new recruits for the sporophytic population. The haploid, straightforward structure of the fern gametophyte is primarily made up of a single layer of cells. Although it lacks sophisticated tissues and organs, it has a tremendous deal of potential to provide important information and understanding about how plants develop. It is an excellent experimental system since it is simple to cultivate and may be handled in a destructive-free manner. For the study of morphogenesis in response to environmental stimuli, the gametophyte of ferns makes an excellent model system (Goller 2007). Additionally, these discoveries offer knowledge to aid in the management, cultivation, and protection of the species (Hanyuan 2003).

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Identification of Novel Bio-Active Compounds from *Kyllinga nemoralis* (Cyperaceae)

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***Abstract*:** *Kyllinga nemoralis* (Hutch and Dalz), a member of the Cyperaceae family, known for its diverse medicinal properties. The aim of the study is to identify and characterize the bioactive compounds present in *K.nemoralis.* Phytochemical screening revealed the presence of phenols, flavonoids, tannins, saponins, steroids, and terpenoids, with high amounts of phenols (109 mg/g) and tannins (59.4mg/g). GC-MS analysis detected 19 compounds, including cyclo octa siloxane hexadecamethyl, 7 methyl bicyclo (3.2.0) hept-3-ene-2-one etc., exhibiting antibacterial, antifungal, and anticancer activities. This study highlights the potential of *K. nemoralis* as a valuable source of natural products for healthcare solutions, contributing to the advancement of medicinal plant-based research.

***Keywords*:** *Kyllinga nemoralis*, Phytochemical compounds, GC-MS analysis

In India, most of the people also depend on traditional herbal medicine systems and still explored number of medicinal plants for their therapeutic activities (Savithramma et al., 2012). Phytochemicals are naturally occurring constituents in medicinal plants, present in all parts which utilizes for defense mechanisms to protect themselves. This specificity was precisely utilized to cure human diseases. Identification of plant phytochemicals through screening is considered to be an effective discovering method. phytochemical constituents of medicinal plants responsible for important physiological functions in living beings (Ballesta et al., 2010). The phytochemicals are mainly two groups i.e., primary and secondary metabolites. Fatty acids, common sugars and proteins are included under primary metabolites. Terpenoids, alkaloids, phenols under secondary metabolites. They show anti-oxidant, free radical scavenging activities, anti-inflammatory, anti-spasmodic, anti-defense, anti-diuretic as anti-diabetic, anti-cancerous, anti-viral and helps plant to fight against pathogenic fungi (Lingarao et al., 2011), also relieve cardio-vascular diseases (Yugandhar and Savithramma 2017).

Considerable research on metabolites has been conducted on dicotyledonous plants, while monocotyledonous plants have received relatively less attention. Cyperaceae family, which comprises a significant group of monocots, remains largely underexplored, with only a handful of studies reported. The densely tangled rhizomes of Cyperaceae species contribute significantly to erosion control and water purification. *K. nemoralis* a perennial herb, also called as water clover or sedge, traditionally used to treat fever, digestive issues and respiratory problems, was investigated for its phytometabolites. As a rich traditional folk medicine, leaves are used to treat snakebites, malarial chills, and diabetes exhibiting analgesic, anti-oxidant, anti-microbial, anti-diabetic and anti-cancer properties. This study aims to investigate the phytochemical properties of *K.nemoralis* focusing on the qualitative and quantitative analysis of secondary metabolites using different polar and no-polar solvents. Additionally, GC-MS analysis was employed to identify the bioactive compounds responsible for its medicinal properties.

MATERIAL AND METHODS

Collection of plant: The plants were collected from the Botanical Garden of Sri Venkateswara University, Tirupati (13.628927°N; 79.419307°E) and identified using dictionary of flowering plants of Chittoor District, Andhra Pradesh and authenticated by Dr.N.Savithramma, Department of Botany, SVU, Tirupati. Leaves of plant are three-angled, 2-15cm long, and 0.2-0.4 cm wide. Stem is triangular, solid, and glabrous. Flower grouped together in terminal head, sessile, white or brown. Petals are absent and fruit is a nut.

Preparation of plant extracts: The plant material along with its roots was washed 2-3 times under tap water followed by distilled water to remove the soil and dirt particles, shade dried and powdered. 5 gms of dried powder was taken and subjected to extraction under with 100 ml of different solvents i.e., distilled water, methanol, chloroform, ethyl acetate and isopropanol. The plant extracts were filtered, stored in the refrigerator for further studies.

Phytochemical screening: Various tests were performed with plant extracts to unveil the metabolites like alkaloids, phenols, flavonoids, saponins, terpenoids (Harbone 1998, Savithramma et al., 2011).

Quantification of secondary metabolites: Phenols,

flavonoids, tannins and steroids of the plant were estimated (Okeke and Ekekwa 2003, De silva et al., 2017)

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: During a GC-MS analysis, the sample is vaporized and carried by an inert gas (like helium) through a column coated with a stationary phase. As the sample travels through the column, its components separate based on their interactions with the stationary phase and their boiling points. The separated components enter the mass spectrometer, where they are ionized typically by electron impact. The ions are then sorted and detected based on their mass to charge ration (m/z). The resulting spectrum provides a unique finger print for identifying and quantifying the compounds. GC-MS analysis of crude methanolic extract was carried out using GC-MS QP2010 Shimadzu (Japan) system comprising a gas chromatograph interfaced to a mass spectrometer. The details of column used, carrier gas and maintenance of column temperature were followed as per the method Konappa et al. (2020).

The plant had shown the presence of many secondary metabolites. More number of compounds were identified in aqueous extract (11) followed by methanol and chloroform. phenols, flavonoids and tannins were recorded in all the solvents. Steroids were not observed in aqueous extract but

Table 1. Preliminary screening of secondary metabolites from *K. nemoralis*

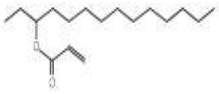
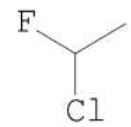
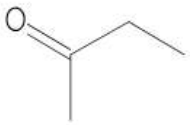
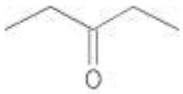
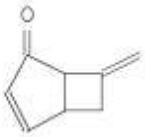
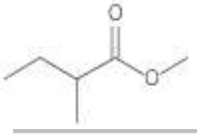
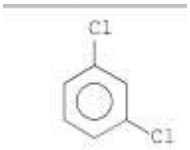
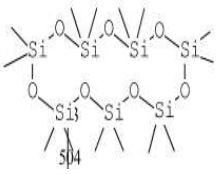
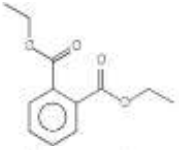
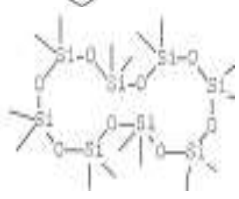
Phytochemical constituent	Aqueous	Methanol	Chloroform	Ethyl Acetate	Isopropanol
Alkaloids	-	-	-	-	-
Steroids	-	+	+	+	+
Phenols	+	+	+	+	+
Flavonoids	+	+	+	+	+
Terpenoids	+	+	+	-	-
Saponins	+	+	+	-	+
Tannins	+	+	+	+	+
Anthroquniones	+	+	-	-	+
Reducing Sugars	+	-	-	-	-
Phlobatannins	+	-	-	-	-
Leucoanthocyanins	+	+	-	+	+
Triterpenoids	+	-	-	+	+
Anthocyanins	+	-	-	-	-
Emodins	-	-	+	-	-

+: present; -: not present

Table 2. Quantitative Estimation of secondary metabolites from aqueous extracts of *K. nemoralis*

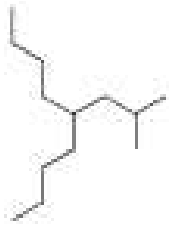
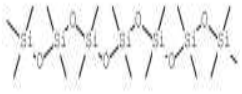
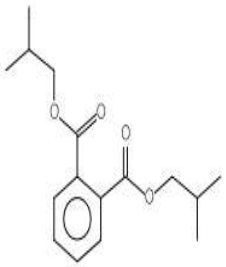
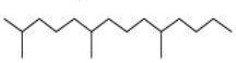
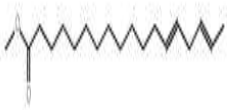
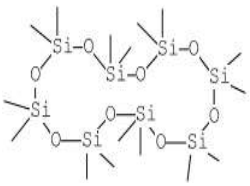
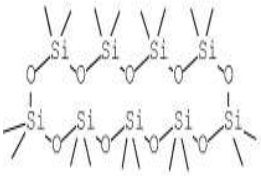
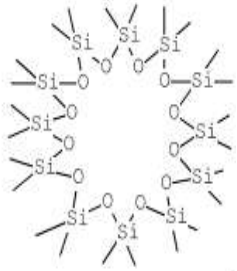
Secondary metabolites	Amount present (mg/g)	Uses
Phenols	109±0.75	Used as fungicide, pesticide, antiseptic and disinfectant, in manufacture of resins; anti-inflammatory, antitoxic, antiviral and anti-microbial, anti-tumor agent. (Shaheen and Savithramma 2022)
Flavonoids	20.8± 0.43	regulation of plant growth, development, disease resistance; anti-inflammatory, analgesic, anti-oxidant, anti-fungal and immune stimulant (Abdallah 2015)
Tannins	59.4± 0.37	Astringent i.e., fasten the wound healing; anti-oxidant, anti-microbial and anti-inflammatory; treats intestinal disorders such as diarrhea and dysentery (Koleckar et al 2008)
Steroids	19.71± 0.29	Has relationship with endocrinal hormones of human beings in their chemical composition (Yaswanthi et al 2024).

Table 3. GC-MS analysis of bioactive compounds found in methanolic extract of *K. nemoralis*

RT value	Name of the compound	Molecular formula	Molecular weight	Peak area	Structure of the compound	Uses
0.122	3Prop-2enoyloxy tetradecane	$C_{17}H_{32}O_2$	248	38		Imparts olfactory properties making it potential ingredient in perfumes or flavouring, its properties make it useful as insecticide or repellant.
1.088	Ethane 1-chloro 1-fluoro	C_2H_4ClF	82	39.6		Used as corrosion inhibitor for steel i.e., binds to water and prevent formation of H bonds with other substances like fatty acids. It is also used as sold catalyst for reaction of chloride with fluorine.
1.629	2-Butanone	C_4H_8O	72	0.8		It is colourless liquid with sweet odour. Used in glues and as cleansing agent. Its long term exposure was reported to have slight neurological,liver, kidney and respiratory effects
1.846	3-Pentanone	$C_5H_{10}O$	86	0.5		It is precursor to Vitamin - E. possess odour like that of acetone, used as solvent in paint.
2.385	7-methylene bicycle (3.2.0) hept-3-ene-2-one	C_8H_8O	120	0.06		Possess biological activities like bacteriostatic, fungistatic and anti-parasitic.
2.515	Butanoic acid 2-methyl-methyl ester	$C_6H_{12}O_2$	116	0.4		It is used as chiral stationary phase in Gas chromatography to allow separation of enantiomers.
5.735	Benzene 1,3-dichloro	$C_6H_4C_{l2}$	146	0.56		It is combustible and toxic to aquatic life.
12.592	Cycloheptasiloxane, tetradcamethyl	$C_{14}H_{42}O_7Si_7$	518	2.6		These are among the wider class of organosilicon. Commonly used in cosmetic industries, to produce deodrats, hairsprays and skin care. It also used in cookware industry and Kitchen utensils, effective industrial cleansing agents in dry cleaning industries. They are good source of electric insulation, low chemical conductivity, low toxicity, hgh gas permeability.
14.461	Diethyl Pthalate	$C_{12}H_{14}O_4$	222	3.6		It is colourless, odourless oily substance used to improve the performance and durability of many products. It is added to plastic polymers to maintain flexibility.
15.542	Cyclo octa siloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	3.26		It exhibits anti-microbial activity especially against 5 pathogensi.e., *P. florescence, P. aeruginosa, S. aureus, Vibreo damsela, Aeromonas hydrophila*

Cont...

Table 3. GC-MS analysis of bioactive compounds found in methanolic extract of *K. nemoralis*

RT value	Name of the compound	Molecular formula	Molecular weight	Peak area	Structure of the compound	Uses
16.077	Nonane 5- (2-methyl propyl)	$C_{13}H_{28}$	184	0.23		Not noticed with any application.
17.815	Heptasiloxane, hexadecamethyl	$C_{16}H_{48}O_6Si_7$	532	2.4		It is used in cosmetics as a film forming polymer. It is also used as antifungal agent by treating many fungal infections. It has alos been shown to have anti-inflammatory properties. This effect may be due to its ability to inhibit prostaglandin synthesis by reversibly binding to enzyme cyclooxygenase.
18.300	1,2 Benzene dicarboxylic acid, bis (2-methyl propyl)	$C_{16}H_{22}O_4$	278	2.9		It is used in adhesives, sealants, paints, coatings, and plastic and rubber products. Some studies have shown that it has potential as a chemoprotective or chemo therapeutic against osteosarcoma.
18.845	Tetradecane 2,6,10-trimethyl	$C_{17}H_{36}$	240	0.7		Anti-bacterial, anti-fungal and nematicidal activity.
20.794	13,16 Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.76		Possess anti-microbial, anti-oxidant and anti-inflammatory activity
21.165	Cyclo 3octa siloxane hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	0.7		It exhibits anti-microbial activity.
28.746	Cyclononasiloxane octadecamethyl	$C_{18}H_{54}O_9Si_9$	666	5.5		It is used as cleansing agent in cosmetics, textiles. Also possess antifungal property, biologically resistant to termites.
30.671	Tetracosamethyl cyclododeca siloxane	$C_{24}H_{72}O_{12}Si_{12}$	888	2.09		Its unique structure gives it excellent lubricating and emollient properties. It has low surface tension which makes it an excellent surfactant reducing interfacial tension between two substances. It is shown to have low toxicity.

detected its presence in all the other extracts of polar and non-polar solvents. Saponins were present in all solvents except ethyl acetate. Leucoanthocyanins, were absent in chloroform extract and emodins were observed only in chloroform extract whereas, reducing sugars and phobia tannins only in aqueous extract. Anthocyanins are seen only in aqueous extract, Triterpenoids are absent in methanol and chloroform extracts and anthraquinones in aqueous, methanol and isopropanol extracts.

Alkaloids are one of the largest group of phytochemicals in plants having amazing effects based on their toxicity against cells of foreign organisms. But alkaloids are absent in *K.nemoralis* whereas present in other members of Cyperaceae which include *Cyperus rotendus, Frimbristylis eragrostis, Fimbristylis monostachya, Paspalidium flavidum*. Phenols, flavonoids, steroids, tannins were present in *K.nemoralis* and found in other members of Cyperaceae i.e.,*Fimbristylis cymosa, Fimbristylis eragostis, Kyllinga triceps, Paspallidum flavidum* but found to be absent in *Cyperus difformis, Scleria lithosperma* (Haribabu and Savithramma 2014).

Among the metabolites identified, phenols (109) were in higher amounts followed by tannins (59.4) from aqueous extracts. Saponins are also present in the plant. Traditionally saponins have been extensively used as detergent as pesticides and molluscides. Saponins have a relation with a hormone oxytocin which is involved in controlling the onset of labour pains in female and the subsequent release of milk. Saponins enhance nutrient absorption and aid in animal digestion. They are bitter in taste and can resume plant palatability. Saponins possess hypocholesterolemic property for the control of high blood lipids in addition to their industrial applications as foaming and surface active agents it also have beneficial health effects (Komuraiah 2014).

GC-MS analysis: Gas chromatography analyses in combination with mass spectra of methanolic extracts were analyzed to identify different phytochemical compounds along with their molecular weight and molecular formula. Nearly 18 compounds are identified with their potential uses (Table 3). Among them cyclo octa siloxane hexadecamethyl, tetradecane 2,6,10-trimethyl, 13, 16 octa decadienoic acid, methyl ester; exhibits anti-bacterial activity. Cyclonona siloxane octa deca methyl, hepta siloxane hexa decamethyl, 7 methyl bicyclo (3.2.0) hept-3-ene-2-one especially possess fungistatic activity. The GC-MS revealed the plant may be used to treat cancer, act against bacteria, fungi and nematodes; as a cleansing agent, also in industrial applications such as chiral stationary phase in paper chromatography.

CONCLUSION

The *Kyllinga nemoralis* is rich in phenols, flavonoids, tannins and steroids. The GC-MS analysis further confirmed the presence of these compounds and provided an information on their chemical structure. The *K. nemoralis* is a rich source of bioactive compounds with pharmacological applications and highlights its potential as a source of novel bioactive compounds.

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Faunal Diversity of Tembao Lake Complex- High-Altitude Wetland In North Sikkim, Eastern Himalaya

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***Abstract*:** High-altitude wetlands in the Himalaya are critical biodiversity rich ecosystems, supporting unique alpine flora and fauna while providing essential ecosystem services to downstream communities. However, due to their remote locations and extreme environmental conditions, these ecosystems remain understudied. This study presents a comprehensive faunal assessment of the Tembao Wetland Complex (TWC) in Sikkim, India, located at an elevation of 5,200 m asl. The study recorded five butterfly species, indicating the presence of diverse insect life adapted to high-altitude conditions. Observation of *Scutiger boulengeri* (Boulenger's Lazy Toad), reaffirmed the significance of TWC as a key breeding ground for high-altitude amphibians. Avian sampling revealed 16 bird species, with evidence of breeding activity in many, underscoring the wetland's role as a crucial avian habitat. Additionally, direct and indirect observations of five mammalian species highlight the area's importance for sustaining wildlife populations in these extreme conditions. Given its ecological significance, we advocate for the designation of TWC as a Ramsar and Heritage Site to ensure the long-term conservation of its biodiversity and to recognize the broader importance of high-altitude wetlands in the Himalayan landscape.

***Keywords*:** Amphibians, Birds, Butterflies, High Altitude Lakes (HALs), Mammals, Wetland ecosystems

Lakes are the major wetland types in the high-elevation region in the Himalaya and those located above 3000 m elevation are generally considered as High-Altitude Lakes (HALs). As per the national wetland inventory and assessment report of the Space Application Centre, Government of India, 4703 HALs occur in the Indian Himalaya, and cover a total area of 126249 hectares. Among the total lakes, few are very large (> 500 ha) or large lakes (100-500 ha), whereas the majority are small (10-25 ha) and very small (<10 ha) of which maximum numbers are of <2.25 ha (Panigrahy et al., 2011). HALs play a significant role in the well-being of the people as well as wetland-dependent biodiversity. In general, these HALs have three important roles- (a) serve as an important habitat for various kinds of organisms, especially birds, amphibians, and insects; (b) due to their aesthetic beauty and pristine nature, these lakes are ecotourism sites of global importance, thus contributing to cultural ecosystem services; and (c) they also form a reservoir for small streams which originates either from nearby glaciers or from the lake itself.

Sikkim state of Indian Union located in the Eastern Himalaya is bestowed with spectacular natural landscapes such as snow-clad mountain peaks (including Mt. Kanchendzonga, 3rd highest mountain peak in the world), high altitude alpine meadows and cold deserts and several wetlands (Acharya and Sharma 2013). Local communities of Sikkim consider all kinds of water bodies as holy places and worship them considering guardian deities of the mountains (Sharma et al., 2012). Lakes form the major components of wetland types. Among all the lakes in the state, 534 lakes have been mapped as HALs which cover an area of 3325 hectares. Sikkim does not have any very large lakes and the majority of the lakes are very small or <2.25 ha in size (Panigrahy et al., 2011).

A few lakes such as Gurudongmar, Tsomgo, Khecheopalri, and TshoLhamo are well known to the people as they are accessible through roads and have become a tourist hub. Scientific studies have been conducted in and around some of these lakes (Subba et al., 2015, Chettri and Acharya 2020). However, information on the geology, environment, and biodiversity of many other lakes, especially those fed by nearby glaciers, is very limited. This is mainly because these lakes are located in high-altitude areas with extremely harsh environmental conditions and are not accessible by road, and also forms a part of sensitive geopolitical boundaries. The shrinkage of glacial areas in high-altitude Himalayan lakes and the expansion of glacial lakes due to climate change pose major threats (Ives et al.,

2010). These changes significantly alter the hydrogeological cycle throughout the catchment area, negatively impacting ecosystems and their services, and increasing the risk of Glacial Lake Outburst Flood (GLOF) events (Milner et al., 2017). Despite their significance as high-priority biodiversity sites and the threats they face, such lake complexes lack proper scientific assessment and policies for their management. An instance of policy gaps is the serious outcomes of the 2023 outburst of high altitude Lhonak Lake in the Sikkim Himalaya. This event occurred as per the predictions and alerts provided by earlier studies (Sattar et al., 2021). Consequences of habitat destruction and biodiversity loss throughout the catchment areas due to this devastating event are entirely unknown due to the unavailability of data before the incident. Therefore, it is essential to establish a fundamental understanding of the biodiversity and function of the ecosystem within the HALs. This information is crucial for developing effective management plans.

The wetland ecosystem offers a variety of essential services that sustain life on Earth and enhance human well-being (Maitry et al., 2023). The Tembao Wetland Complex (TWC), located in the high-altitude alpine region of Mangan district, northern Sikkim, comprising Tembao Lake, glacial-fed Tembao Chu, and smaller high-altitude lakes, remains scientifically unexplored (ENVIS 2007). Despite its ecological and geological significance, TWC faces threats such as catchment degradation, wetland shrinkage, and traditional grazing, necessitating urgent conservation efforts. The Sikkim government aims to integrate TWC with nearby wetlands for better management and potential inclusion in the National Wetland Conservation Programme, but baseline biodiversity data is essential for conservation planning and Ramsar designation. To address these data gaps, we conducted a rapid biodiversity assessment of TWC's faunal components in August 2022, focusing on butterflies, amphibians, birds, and mammals. These taxa serve as indicators of habitat quality and climate change, representing key species of the high-altitude alpine ecosystem.

MATERIAL AND METHODS

Study area: The Tembao Wetland Complex (27^{O} 54' 51" to 27^{O} 54' 37" and 88^{O} 43' 37" to 88^{O} 47' 24" E) is situated at the northern frontiers of Lachung-Yumthang valley in Mangan district of Sikkim, India (Fig. 1, 2). The elevation of the entire complex ranges between 4400 to 5200 meters above sea level (m asl), and has an area of approximately 15 hectares. The complex is fed by a river originating from the glacial Tembao Lake at around 5200 m asl elevation. The average annual temperature of the area remains close to sub-zero and the average precipitation is below 500 mm. The vegetation type is representative of the alpine tundra far above the tree line where grasses and herbs are more dominant. The TWC is a unique high-altitude ecosystem near the tri-junction of India, China, and Bhutan, home to specialized plant species such as *Meconopsis*, *Saussurea*, *Rheum nobile*, and others, thriving in extreme climatic conditions (Acharya and Sharma 2012). While largely free from tourism and development pressures, the region experiences seasonal cattle grazing, whose impact on biodiversity remains debated. However, climate change poses a major threat, with rising temperatures accelerating glacial retreat, increasing the risk of GLOFs, and causing catchment degradation, highlighting the need for continuous monitoring and transboundary conservation efforts (Kaltenborn et al., 2010; Milner et al., 2017, Veettil and Kamp 2019, Liu and Chen 2023).

Taxon sampling

Butterflies: During the study, transect walks (along existing trails) and opportunistic survey methods was used to assess the species richness and abundance of butterflies in the TWC. In total, four transects, each 1 km in length, were identified and selected, maintaining a minimum distance of 1 km between consecutive transects. Sampling took place from morning to afternoon hours (9:00-14:00 hrs), coinciding with peak butterfly activity during foraging, mating, and patrolling behaviors. Butterflies observed within 5 m distance on either side of the trail/transect were identified, and their species name and abundance were recorded. Comprehensive field guides such as Haribal (1992) and Kehimkar (2016) were used to identify all butterflies recorded during sampling and opportunistic observations. For butterflies posing identification challenges in the field, photographs were taken and identification was performed at a later stage when resources were accessible following field guides (Haribal 1992, Kehimkar 2008, 2016, Smetacek 2016) and online repository (Kunte et al., 2022).

Amphibians: Visual Encounter Survey (VES) method, as outlined by Heyer et al. (1994), was used for sampling amphibians. VES was conducted extensively across all feasible habitats and microhabitats within the TWC, with particular focus on water bodies such as ponds, ditches, and rivers. Visual scanning, along with the careful manipulation of boulders, pebbles, plant leaves, and mud, was employed to uncover amphibians. Sampling occurred between 10:00 hrs and 16:00 hrs over five consecutive days, amounting to a total of 30 man-hours of VES effort. During the VES, species encountered above the surface were recorded without disturbing their microhabitats. Furthermore, following species identification, various parameters including the

number of individuals, tadpoles, eggs, encounter time, and precise location (GPS coordinates) were recorded during the study.

Birds: The opportunistic sampling method was used to assess the bird community in the TWC, North Sikkim, following the protocol outlined by Vaz et al. (2020). The sampling consisted of walking through existing trails within the lake complex during both morning (05:00-09:00 hrs) and evening hours (14:00-16:00 hrs), during which we recorded all bird species observed or heard. The bird sampling was conducted over five consecutive days, covering various parts of the TWC to ensure comprehensive coverage without repeating surveyed areas on subsequent days. Birds were observed using binoculars and identified visually. In cases where instant identification was not feasible, photographs were taken whenever possible, and identification was subsequently performed by referencing the Merlin Bird ID app and the field guide (Grimmet et al., 2019).

Mammals: Mammal sampling was conducted utilizing camera traps, a non-invasive technique, in conjunction with direct observation. A total of eight camera traps (Cuddeback C2 Xchange Infrared Trail Game Camera) were strategically positioned in areas exhibiting frequent animal activity, discernible from direct observations and signs such as tracks, pugmarks, hoofs, scat, faeces, and digging signs to maximize chances for photo capture (Sathyakumar et al., 2011). The cameras were configured in wide detection mode

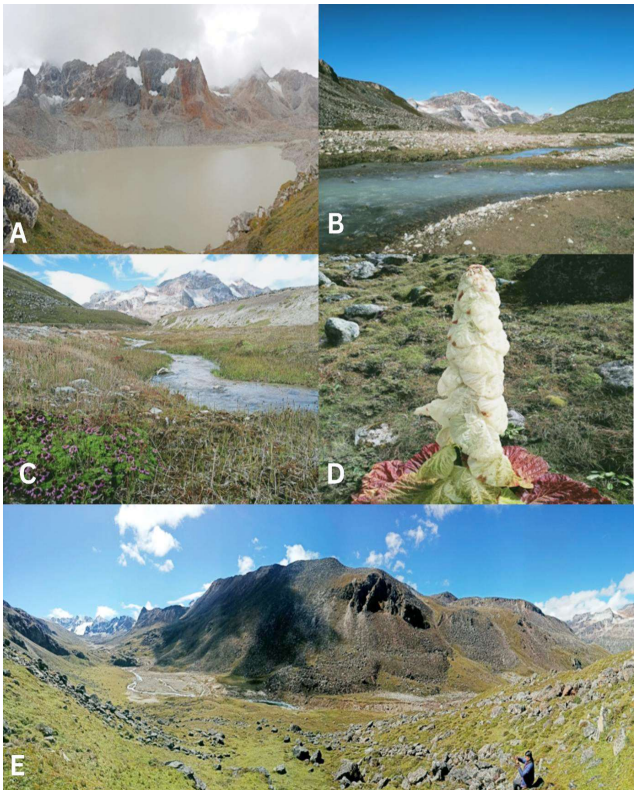

Fig. 2. A: Tembao Lake, B-C: Glacial-fed River and stream flowing through TWC valley, D: *Rheum nobile*, a giant herbaceous plant found in the TWC area, E: Panoramic view of TWC located in northern Sikkim, Eastern Himalaya

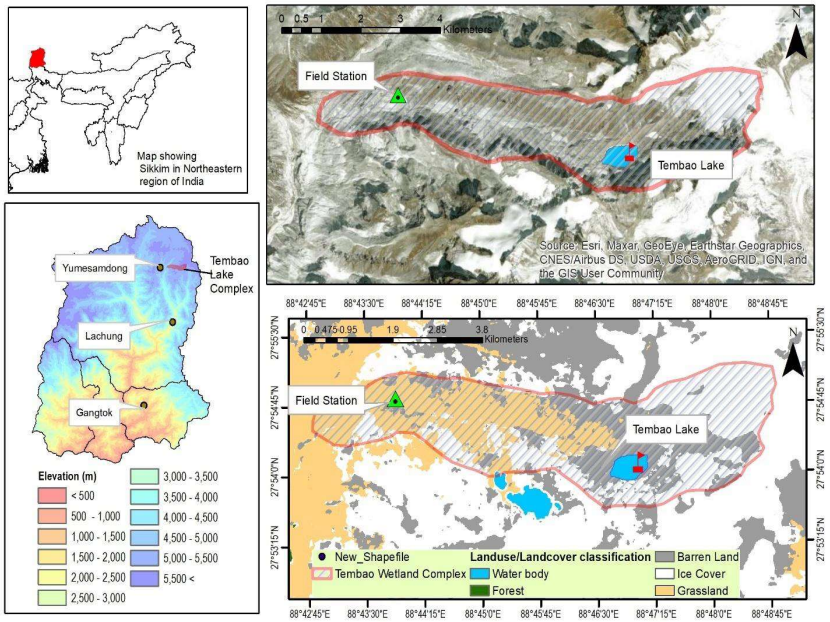

Fig. 1. Location of Tembao Wetland Complex in North Sikkim. The green legend with the black dot in the center indicates the field station and the red rectangular legend with the red flag depicts the location of Tembao Lake

to ensure the capture of smaller and fast-moving animals (Meek et al., 2014, Gillespie et al., 2015). A minimal time lapse between camera triggers, set to FAP (as fast as possible), with burst mode capturing three images per encounter, was implemented. The cameras remained deployed in selected locations covering the areas of the TWC for durations ranging from two to four consecutive days, without the use of baits to attract animals. Simultaneously, direct observations were conducted through walk along existing trails and surrounding areas. This involved approximately 40 km (equivalent to approximately 20 man-hours) of on-foot transects along paths, and approximately 12 km (equivalent to approximately 10 man-hours) of exploration around the vicinity. Species encountered during the study period were identified with the help of field guides such as "Mammals of India" by Menon (2014), and through consulting with domain experts.

RESULTS AND DISCUSSION

Butterflies: During the study period, 19 butterfly individuals representing five species, with three species belonging to the family Nymphalidae and one species each from Papilionidae and Pieridae were recorded (Table 1). The most abundant species was the Himalayan Dark Clouded Yellow (*Colias fieldii fieldii*), followed by the Darjeeling Gem Silverspot (*Issoria gemmata gemmata*), Ladakh Tortoiseshell (*Aglais ladakensis*), Himalayan Tortoiseshell (*Aglais caschmirensis aesis*), and the Common Blue Apollo (*Parnassius hardwickii*). The findings represent a significant portion of the expected biodiversity in the Trans-Himalayan Wildlife Corridor (TWC) and likely do not capture the full extent of the alpine butterfly fauna, particularly species with cryptic behaviors or those exhibiting seasonal fluctuations. Rapid assessment surveys, though valuable for providing quick biodiversity insights, have inherent limitations that may have led to under-detection. The survey recorded only 16% of the 31-butterfly species expected at this elevation (>4000 m asl.) (Haribal 1992, Kehimkar 2016), with several missing species from diverse groups such as Apollos (*Parnassius acco, Parnassius acco hunningitoni, Parnassius acdestis, Parnassius hardwickii, Parnassius simo*), Argus (*Paraoeneis palaearcticus sikkimensis, Paraoeneis pumilus bicolor*), Tibetan Blackvein (*Mesapia peloria*), Blues (*Albulina arcaseia, Albulina asiatica*), Nepalese Large Cabbage White (*Pieris brassicae nepalensis*), and Fritillaries (*Issoria issaea, Melitaea arcesia sikkimensis, Melitaea arcesia thibetana*). 32% of the unrecorded species have restricted geographic ranges (<1000 meters), suggesting their strong dependence on specific alpine habitat features or microclimates (Kehimkar 2016). Despite these limitations, the presence of the observed species underscores the resilience and adaptability of butterflies in this high-altitude ecosystem.

Herpetofauna: We recorded single amphibian species, *Scutiger boulengeri*, commonly known as Boulenger's Lazy Toad or Xizang Alpine Toad. A total of nine adults were observed in various ditches and ponds around the Tembao Wetland Complex, along with 336 tadpoles at different developmental stages. As this species requires stable and calm aquatic environments for metamorphosis, the smaller ponds within the complex serve as critical breeding grounds. Such ponds are particularly significant as the main river originating from Tembao Lake flows at high velocity due to the steep terrain. Conservation of these small water bodies is therefore vital for the long-term survival and reproduction of *Scutiger boulengeri*. The species holds the record for the highest-altitude toad, having been documented at 5270 m asl. at Gurudongmar Lake in North Sikkim (Subba et al., 2015). Its detection in Sikkim marked the first-ever record of this species in India, extending its known distribution beyond northwestern Nepal, southeastern Qinghai, eastern and southern Xizang, southern Gansu, and northwestern Sichuan in western China (Ohler et al., 2011). The presence of this high-altitude specialist further highlights the ecological uniqueness of the TWC and underscores the importance of conserving its diverse microhabitats.

Birds*:* A total of 142 individual birds from 16 species across nine families were documented during the study period (Table 1). The families Motacillidae and Muscicapidae exhibited the highest species richness (three species each), while Accipitridae, Columbidae, Passeridae, and Turdidae were represented by only one species each. The Red-billed Chough (*Pyrrhocorax pyrrhocorax*) was the most abundant species (29 individuals), followed by the Alpine Accentor (*Prunella collaris*, 25 individuals) and the White-winged Redstart (*Phoenicurus erythrogastrus*, 22 individuals). In contrast, the Bearded Vulture (*Gypaetus barbatus*), a Near Threatened species on the IUCN Red List, was the least encountered species, with only a single individual recorded. Habitat utilization patterns revealed three primary categories, with rocky area species exhibiting the highest richness (56.25%), followed by wetland-dependent species (25%) and shrubland species (18.75%).

Notably, 87.50% of the species recorded during the study were altitudinal migrants, with only 12.50% being resident species. The breeding elevation ranges of the recorded species, assessed following Grimmett et al., (2019) and the IUCN Red List (2024), spanned from 1800 m to 6200 m asl., with 81.25% of species having breeding ranges within the TWC elevation range (4400–5200 m asl) (Fig. 3). Species such as the White-rumped Snowfinch (*Montifringilla*

taczanowskii), Grandala (*Grandala coelicolor*), and Alpine Accentor have breeding distributions closely aligned with TWC's elevation range, emphasizing the importance of this wetland as breeding ground for high-altitude avifauna. Although the species recorded represent only 2.79% of the total bird species documented in Sikkim, they account for 9.47% of temperate species recorded in the state (Acharya and Vijayan 2011). Additionally, 43.75% of the birds recorded in TWC are biome-restricted species associated with the Eurasian high montane (alpine and Tibetan) biome, including the Alpine Accentor, Alpine Chough (*Pyrrhocorax graculus*), Common Rosefinch (*Carpodacus erythrinus*), Grandala, Robin Accentor (*Prunella rubeculoides*), Rosy Pipit (*Anthus roseatus*), and Snow Pigeon (*Columba leuconota*) (Ganguli-Lachungpa et al., 2011). The microhabitat around the wetlands supports a diverse range of bird species (Jamakhandi and Kadadevaru 2024). The prevalence of rocky area specialists and altitudinal migrants, along with the significant number of

Table 1. Different faunal species in the Tembao Wetland Complex in North Sikkim, Eastern Himalaya

Family	Species name	Common name	Abundance
Phylum Arthropoda Class Insecta Order Lepidoptera			
Family Papilionidae	*Parnassius hardwickii* (Gray 1831)	Common Blue Apollo	1
Family Nymphalidae	*Issoria gemmata* (Butler, 1881)	Gem Silverpsot	5
	Aglais ladakensis (Moore, 1878)	Ladakh Tortoiseshell	4
	Aglais caschmirensis (Kollar,[1844)	Indian Tortoiseshell	3
Family Pieridae	*Colias fieldii* Ménétriés, 1855	Dark Clouded Yellow	6
Phylum Chordata Class Amphibia			
Family Megophryidae	*Scutiger boulengeri* (Bedriaga 1898)	Boulenger's Lazy Toad	9 adults, 336 tadpoles
Class Aves			
Family Accipitridae	*Gypaetus barbatus* (Linnaeus 1758)	Bearded Vulture	1
Family Corvidae	*Pyrrhocorax graculus* (Linnaeus 1766)	Alpine Chough	3
	Pyrrhocorax pyrrhocorax (Linnaeus 1758)	Red-billed Chough	29
Family Columbidae	*Columba leuconota* (Vigors 1831)	Snow Pigeon	6
Family Prunellidae	*Prunella collaris* (Scopoli 1769).	Alpine Accentor	25
	Prunella rubeculoides (Moore 1854)	Robin Accentor	6
Family Muscicapidae	*Phoenicurus frontalis* (Vigors 1832)	Blue-fronted Redstart	4
	Chaimarrornis leucocephalus (Vigors 1831)	White-capped Water Redstart	5
	Phoenicurus erythrohastrus (Güldenstadt 1775)	White-winged Redstart	22
Family Motacillidae	*Motacilla flava* (Linnaeus 1758)	Yellow Wagtail	2
	Anthus trivialis (Linnaeus 1758)	Tree Pipit	4
	Anthus roseatus (Blyth 1847)	Rosy Pipit	8
Family Turdidae	*Grandala coelicolor* (Hodgson 1843)	Grandala	12
Family Fringillidae	*Leucosticte nemoricola* (Hodgson 1836)	Plain Mountain Finch	7
	Carpodacus erythrinus (Pallas 1770)	Common Rosefinch	4
Family Passeridae	*Montifringilla taczanowskii* (Prjevalsky 1876)	White-rumped Snowfinch	4
Class Mammalia			
Family Bovidae	*Pseudois nayaur* (Hodgson 1833)	Blue Sheep	65
Family Sciuridae	*Marmota himalayana* (Hodgson 1841)	Himalayan Marmot	6
Family Canidae	*Vulpes vulpes* (Linnaeus 1758)	Red Fox	1
Family Mustelidae	*Mustela altaica* (Pallas 1811)	Altai Weasel	3
Family Ochotonidae	*Ochotona macrotis* (Günther 1875)	Large-eared Pika	2

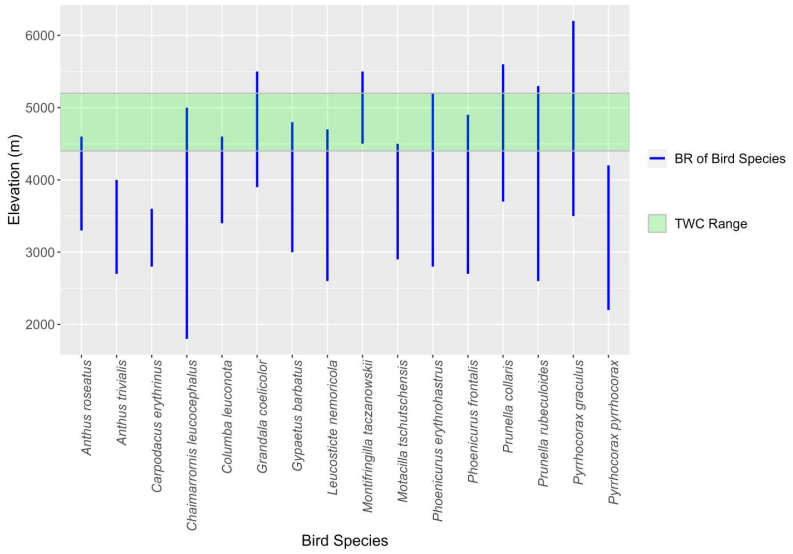

Fig. 3. Elevation BR (breeding range) of bird species relative to TWC (Tembao Wetland Complex) elevation range

species that breed within the TWC, underscores the ecological significance of this wetland complex in supporting specialized high-altitude bird communities.

Mammals: Five mammal species from five families were recorded. Blue Sheep (*Pseudois nayaur*) was the most frequently observed species, with three herds comprising 65 individuals (6, 25, and 34), detected both through direct observation and camera traps (Table 1). Blue Sheep typically inhabit elevations between 2500 and 5500 m asl. (Harris 2014a), and the present study recorded them at over 5000 m asl., making it the highest documented occurrence in the Sikkim Himalaya. This exceeds previous records from the Khangchendzonga Biosphere Reserve (>4000 m asl.; Sathyakumar et al., 2011) and the Tso Lhamo Plateau (>4300 m asl.; Chanchani et al., 2010). The topography of the TWC, characterized by gentle slopes and minimal rock cover, provides a prime habitat for this species. Additionally, Himalayan Marmots (*Marmota himalayana*) were recorded through six direct sightings and one camera trap, with over 15 burrows identified. These burrows were primarily found near sunny slopes and water sources, consistent with previous studies (Aryal et al., 2015, Shrestha 2016, Wang and Hou 2021). Marmots coexist with Yaks, benefiting from grazing activity that promotes the growth of young plants by clearing dead vegetation (Nikol'skii and Ulak 2006), though guarding dogs used by herders may influence their activity (Poudel et al., 2015). Red Fox (*Vulpes vulpes*), typically found up to 4500 m asl. (Hoffmann and Sillero-Zubiri 2021), was recorded at over 5000 m asl., suggesting an upward range extension that warrants an update to its distribution. Similarly, Altai Weasel (*Mustela altaica*), recorded at three locations, is usually found between 80 and 4900 m asl (Abramov 2016). However, the absence of agriculture and regulated grazing under the traditional Dzumsa system (Acharya and Sharma 2012) minimizes the threats faced by this species in the study area, and its presence above 5000 m asl. further suggests a potential range expansion. Lastly, Large-eared Pika (*Ochotona macrotis*) was observed in rocky habitats, which provide ideal conditions due to moraines formed by past glaciations. This species typically inhabits elevations between 2300 and 6400 m asl. (Smith and Lissovsky 2016), and no immediate threats to its population were observed.

The recorded mammal species represent approximately 28% of the potential mammalian diversity in the area based on geographical distribution and literature reviews. The presence of healthy Blue Sheep populations suggests the potential occurrence of apex predators such as the Snow Leopard (*Panthera uncia*; Aryal et al., 2016), emphasizing the ecological significance of the region. Furthermore, based on their known distributional ranges, other mammal species likely to inhabit the Tembao Wetland Complex include Plateau Pika (*Ochotona curzoniae*, 3000–5000 m asl.; Smith and Liu 2019), Royle's Pika (*Ochotona roylei*, 2400–5400 m asl.; Bhattacharya and Dahal 2021), Woolly Hare (*Lepus oiostolus*, 2500–5400 m asl.; Smith and Johnston 2019), Eurasian Lynx (*Lynx lynx*, 0–5500 m asl.; Breitenmoser et al., 2015), Dhole (*Cuon alpinus*, 0–5300 m asl.; Kamler et al., 2015), Tibetan Fox (*Vulpes ferrilata*, 2500–5200 m asl.; Harris 2014b), Brown Bear (*Ursus arctos*, 0–5000 m asl.; McLellan et al., 2017), Siberian Weasel (*Mustela sibirica*,

0–4875 m asl.; Acharya et al., 2016), Kiang (*Equus kiang*, 2700–5400 m asl.; Shah et al., 2015), Alpine Musk Deer (*Moschus chrysogaster*, 2000–5000 m asl.; Harris 2016), Tibetan Gazelle (*Procapra picticaudata*, 3000–5750 m asl.; IUCN SSC Antelope Specialist Group 2016), Himalayan Tahr (*Hemitragus jemlahicus*, 1500–5200 m asl.; Ale et al., 2020), and Argali (*Ovis ammon*, 400–5500 m asl.; Reading et al., 2020). The potential presence of these species further highlights the biodiversity and conservation importance of the Tembao Wetland Complex.

The present findings underscore the ecological significance of the Tembao Wetland Complex (TWC) as a crucial habitat for diverse faunal groups, highlighting their reliance on specific alpine habitat features and microclimates. The absence of several expected species during our survey emphasizes the need for targeted research spanning different seasons, employing long-term monitoring to enhance baseline biodiversity data. Future studies should incorporate comprehensive survey methods, including microhabitat assessments, intensive camera trapping for elusive and nocturnal species, and focused conservation efforts such as protecting smaller ponds and managing habitat alterations. Additionally, addressing climate change impacts through mitigation strategies and fostering community engagement will be essential for safeguarding TWC's unique biodiversity. Implementing these measures will provide a robust framework for conservation, ensuring the ecological integrity of this high-altitude ecosystem for future generations.

CONCLUSIONS

The Tembao Wetland Complex (TWC) is a unique high-altitude glacial-fed ecosystem supporting rare and cold-adapted species, emphasizing its ecological and conservation significance. Despite the study's seasonal limitations, the presence of breeding habitats for rare fauna highlights the wetland's importance for biodiversity retention and conservation. To safeguard this fragile ecosystem, we recommend its designation as a “Ramsar Site” based on Criteria 1, 2, 3, 4, and 9, which would be a milestone for wetland conservation in Sikkim. Additionally, developmental activities, including road construction, should be strictly prohibited, as increased accessibility could disturb the habitat and threaten rare species. If necessary, a walking trail using eco-friendly methods may be constructed for herders and trekkers to minimize environmental impact. Furthermore, considering its ecological and cultural significance, TWC can also be declared a “Heritage Site” under the Indian Biological Diversity Act, 2022, and the Sikkim Biodiversity Rules, 2006, ensuring long-term protection of this fragile alpine ecosystem.

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AUTHOR'S CONTRIBUTION

BKA initiated and conceptualized the study. BKA, IKC, BT, RT, TN, and SD contributed to field data collection, data analyses and writing of the original manuscript draft. All authors contributed to the editing and final draft of the manuscript.

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Nutritional Composition, Minerals and Vitamins Analysis of Brussels Sprouts Genotypes: A Comparative Study across Diverse Growing Environments

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***Abstract*:** This study examined the nutritional composition, mineral and vitamins analysis of Brussels sprouts genotypes cultivated under diverse environmental conditions. Four genotypes Hild's Ideal, Long Island Improved, Urja and Franklin F_1 were evaluated under open and protected environment. There were statistically significant variations in nutrient composition between cultivation environments. Brussels sprouts were characterized as a rich source of minerals including iron, magnesium, phosphorus, copper, potassium, zinc and manganese. The highest moisture (87.35%) and crude fat (1.53%) were recorded in Franklin F_1while the genotype Urja exhibited the highest crude fibre (3.59%) under polyhouse conditions. Long Island Improved genotype demonstrated the highest total carbohydrates (31.79%) and food energy (192.26Kcal/100g) under open field conditions. Brussels sprouts also found to be rich source of Vitamins C, E, K and A. This study highlights the critical influence of environment conditions on the nutritional and functional quality of Brussels sprouts and provides insights into optimizing cultivation strategies to enhance their health benefits.

Keywords: Brussels sprouts, Proximate composition, Minerals, Vitamins, Protected condition

Brassica oleracea var. *gemmifera*, commonly known as brussels sprouts, is a cold-season vegetable cultivated for its edible "buds or sprouts," which resemble miniature cabbages. This hardy and slow-growing plant belongs to the genus *Brassica* and the family Brassicaceae sharing a close phylogenetic relationship with other crops like cauliflower, broccoli, kale, and collards. These crops are widely recognised for their high nutritional value and versatility in culinary applications. In brussels sprouts the buds form in the leaf axils, the angles between the leaves and the stem, with the development initiating at the base of the stem and progressing upward. Each sprout/bud develops in the axil of a leaf, resulting in the characteristic arrangement of multiple small, round buds along the stem (Kumar et al., 2014). In recent year the cultivation of Brussels sprouts has gained popularity among Indian growers, especially in and around major metropolitan areas and hill stations, driven by growing demand from hotels and tourists. In India, Brussels sprouts are primarily grown in the Kodaikanal and Nilgiri hills of Tamil Nadu and in parts of Maharashtra. This crop is recognized for its distinctive botanical characteristics within the broader *Brassica oleracea* species (Tewari et al., 2020). Nutritionally, Brussels sprouts are very rich. The brussels sprouts rank among the top 20 most nutritious foods according to their aggregate nutrient density index score, based on vitamin, mineral, and phytonutrient content relative to caloric content, which might increase interest in them (Hwang 2017). Despite the crop's ability to grow in a wide range of soil types, sandy loams and silt loam soils perform best. The pH of the soil is another important factor that determines the fate of a crop for a winter crop. Of these, sandy loam soil is known to be a better option for an early crop. Tewari et al. (2020) suggested in range of 5.8 and 7.2, while Kumar et al. (2014) propose a narrower range of 6.0 to 6.8. But in Himachal Pradesh Brussels sprouts is non traditional vegetable crop. No systematic research has been conducted on the evaluation of Brussels sprouts in Himachal Pradesh, despite the state having favourable agro-climatic conditions for their cultivation. The current studies aim to promote high-value Brussels sprouts by examining the proximate composition, minerals and vitamins analysis across a wide range of environmental conditions. Analyzing the proximate composition, minerals and vitamins content of Brussels sprouts will provide valuable insights into their nutritional profile.

MATERIAL AND METHODS

Plant materials: The genotype of Brussels sprouts variety Hild's Ideal was procured from ICAR- Indian Agricultural Research Institute, Regional Station Katrain, Kullu (HP). Genotypes of Long Island Improved and Franklin F_1were procured from open market of Distt. Kangra (HP), whereas the genotype of Urja variety were procured from KISANeSTORE 2, Vadodara, Gujarat state, India.

Experimental site: Field experiment was laid out at the Agricultural Research Farm of the Krishi Vigyan Kendra, (CSKHPKV) Kangra, District. Kangra (HP), India, during *rabi* season of (2022-2023 & 2023-2024) from September to February. The geographically Agricultural Research Farm of the Krishi Vigyan Kendra is situated at 32.107753° latitude, 76.262736° longitude (Fig. 1).

Sample preparation: Samples were collected from experiment site at the Agricultural Research Farm of the Krishi Vigyan Kendra, Kangra Himachal Pradesh. Brussels sprouts were harvested at physiological or horticultural maturity. Immediately after harvesting, fresh sprouts were cut into small strips and shade dried using the filter papers. The dried samples were finely ground with a mortar and pestle. The dried powder of the brussels sprouts was stored at room temperature until its application for nutritional analysis.

Proximate composition: The proximate compositions of Brussels sprouts were determined in triplicate using standardized protocols of the Association of Official Analytical Chemists (AOAC 2005, AOAC 2000) methods with minor modifications, which are well-regarded for their accuracy and reliability. The moisture content of fresh Brussels sprouts was evaluated by oven drying method. The ash content was determined by using combustion method with muffle furnace at 550°C, Soxhlet extraction method with n-hexane was used for crude fat. Crude fibre was estimated by muslin cloth method. Kjeldahl method was used for the determination of crude protein content. Total carbohydrates content was determined by Phenol sulphuric acid method (Dubois et al., 1956).

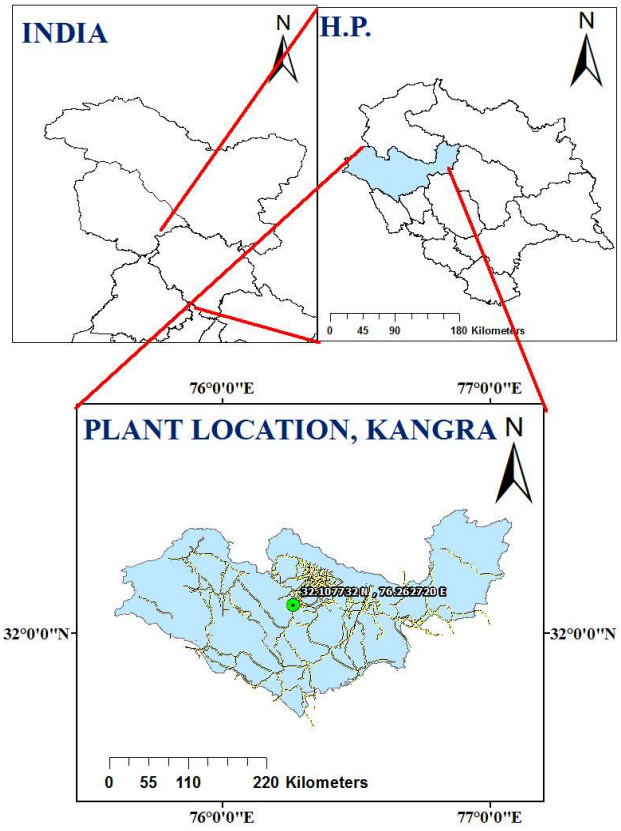

Fig. 1. Experiment site at KVK, Kangra (H.P.)

Food energy: The energy value of Brussels sprouts based on their protein, carbohydrate and fat content was determined according to the conversion factor using the following equation (Verma and Srivastat 2017):

Energy (Kcal/100g) = (%CP × 4) + (%F × 9) + (%CHO × 4)

Where CP = crude protein; F = fat; and CHO = carbohydrate

Mineral analysis: Atomic absorption spectrophotometer was used to estimate macro and micro minerals using the method by Renuka et al. (2016).

Vitamin analysis: The dry powder of various Brussels sprouts genotypes were analyzed for their vitamin content using by high performance liquid chromatography (HPLC).

Statistical analysis: The raw data was statistical analysis using IBM-SPSS.

RESULTS AND DISCUSSION

Proximate composition: The nutritional compositions of the different genotypes of Brussels sprouts are statistically significant different (Table 1). The proximate composition including moisture content under open environment showed highest moisture content (85.86%) in genotype Long Island Improved which was statistically higher than other genotypes and lowest (84.89%) in Franklin F_1 genotype whereas under polyhouse highest moisture content (87.35%) was observed in genotype Franklin F_1 and lowest (85.95%) in Hild's Ideal genotype. The moisture is an essential variable in food processing as well as testing. The moisture content value of brussels sprouts is consistent lower (Mark et al., 2013) with moisture value of 88.4%, 88.2%, and 96% in green cabbage, broccoli, and lettuce. Hanif et al., (2006) also reported high moisture content. The presence of more than 15% moisture content in fruits and vegetables promote microbial activity throughout storage, however a rigid as well as compact head of brussels sprouts make it a more challenging for microorganisms to access, resulting in longer shelf life. Highest ash content (9.63%) was in genotype Urja and lowest (6.4%) in Hild's Ideal genotype under open environment whereas under protected environment highest ash content (10.20%) was Long Island Improved and lowest (8.95%) in Franklin F_1 . Doniec et al. (2022) reported less ash content in brussels sprouts raw material as well as after thermal treatments. Kmiecik et al. (2007) observed the ash

content 7.01g/100g in brussels sprouts and 8.60g/100g in broccoli. The highest crude fibre content was Urja under both environmental conditions which was followed by Long Island Improved under protected conditions and Franklin F_1 under open environment. The highest fat content (1.35%) was observed in genotype Urja under open field whereas under protected environment highest fat content (1.53%) was in Franklin F_1. The crude fat content in both conditions were higher than reported in brussels sprouts (Anonymous 2011). Under open environment highest crude protein content (14.45%) was in Hild's Ideal which was statistically higher to other genotypes except Long Island Improved (14.25%) and lowest (12.25%) in Urja whereas under polyhouse highest crude protein content (17.25%) in Urja and lowest (13.35%) in s Long Island Improved. The highest carbohydrates content was observed in Long Island Improved genotype which was followed by Franklin F_1 genotype in both environmental conditions. Hanif et al. (2006) reported 4.8% carbohydrate content and Ogbede et al. (2015) 4.52% carbohydrate content in *Brassica oleracae* var. *Capitata* L.

Food energy: Food energy content ranges from 156.08Kcal/100g to 192.26Kcal/100g and 167.06 to 188.45Kcal/100g under open field and polyhouse respectively. The total energy content ranges from 24 to 40KCal/100g in various *Brassica* vegetables (Anon 2011) while 248.8 to 307.1KCal/100g in some leafy vegetables (Isong et al., 1999). The observed differences in proximate composition and food energy could be attributed to environmental factors, varietal difference, bioavailability, physical properties of the soil and other factors (Soetan et al., 2010).

Mineral analysis: The brussels sprouts are excellent source of all the necessary dietary minerals. There was significant variation of the mineral analysis of different genotypes of brussels sprouts grown under open as well as protected environment (Table 2). The minerals contents phosphorus (0.15 to 0.18%), Potassium (0.84 to 1.20%) and magnesium (0.16 to 0.21%) were observed under open environment and under polyhouse condition (phosphorus 0.14 to 0.19%, potassium (0.20 to 1.02%) and Magnesium (0.25 to 0.31%). Lewu & Kambizi (2015) reported phosphorus, potassium and magnesium content were 0.56, 3.20 and 0.26% respectively in *Brassica napus* and 0.56, 2.81and 0.90% respectively in *Brassica oleracea*. Emebu and Anyika (2011) reported magnesium content (6.69mg/100g) in kale. Manganese content varies from 22.15 to 29.35 and 22.95 to 28.15mg/kg under open field and polyhouse respectively. The significantly higher manganese content (29.35mg/kg) was observed in Urja variety under open field which was followed by Long Island Improved (28.15mg/kg) under protected

Table 1. Proximate composition and food energy of different genotypes of brussels sprouts grown under open and protected environment

Proximate composition (%)	Open environment				Protected environment			
	Hild's ideal	Long Island improved	Urja	Franklin F_1	Hild's ideal	Long Island improved	Urja	Franklin F_1
Moisture	84.92^{a}	85.86^{b}	85.02^{a}	84.89^{a}	85.95^{a}	86.27^{b}	86.35^{b}	87.35^{c}
Ash	6.41^{a}	8.93^{c}	9.63^{d}	7.76^{b}	9.70^{b}	10.20^{c}	9.78^{b}	8.95^{a}
Crude fibre	2.15^{a}	2.68^{b}	3.53^{d}	3.11^{c}	2.99^{a}	3.14^{a}	3.59^{b}	3.06^{a}
Crude fat	1.15^{b}	0.90^{a}	1.35^{c}	1.05^{ab}	1.13^{a}	1.26^{a}	1.21^{a}	1.53^{b}
Crude protein	14.45^{b}	14.25^{b}	12.25^{a}	12.35^{a}	14.35^{b}	13.35^{a}	17.25^{c}	14.53^{b}
Carbohydrates	23.08^{a}	31.79^{d}	23.73^{b}	29.10^{c}	24.86^{a}	30.03^{d}	27.13^{b}	28.58^{c}
Energy (KCal/100g)	160.49^{b}	192.26^{d}	156.08^{a}	175.58^{c}	167.06^{a}	184.00^{b}	188.45^{d}	186.26^{c}

Means with different letters within the same row shows significant difference ($p<0.05$)

Table 2. Mineral analysis of brussels sprouts grown under open and protected environment

Genotypes	Open environment							Protected environment						
	Macro elements (%)			Micro elements (mg/ kg)				Macro elements (%)			Microelements (mg/ kg)			
	P	K	Mg	Mn	Fe	Cu	Zn	P	K	Mg	Mn	Fe	Cu	Zn
Hild's Ideal	0.18^{b}	1.17^{c}	0.19^{b}	22.15^{a}	106.15^{a}	16.10^{d}	40.90^{c}	0.14^{a}	1.02^{d}	0.30^{bc}	23.65^{c}	149.25^{a}	10.45^{a}	40.00^{c}
Long Island Improved	0.16^{b}	0.84^{a}	0.16^{a}	25.45^{c}	452.55^{d}	13.65^{b}	33.15^{a}	0.19^{c}	0.81^{c}	0.29^{b}	28.15^{d}	315.55^{c}	13.20^{b}	35.65^{b}
Urja	0.14^{a}	1.03^{b}	0.17^{a}	29.35^{d}	128.30^{b}	14.85^{c}	35.40^{b}	0.15^{ab}	0.20^{a}	0.25^{a}	23.05^{b}	243.25^{b}	14.85^{c}	34.95^{a}
Franklin F_1	0.15^{a}	1.20^{c}	0.21^{c}	23.10^{b}	318.25^{c}	13.25^{a}	43.20^{d}	0.16^{b}	0.71^{b}	0.31^{c}	22.95^{a}	323.50^{d}	18.00^{d}	41.05^{d}

Means with different letters within the same row shows significant difference ($p<0.05$)

conditions. Lewu and Kambizi (2015) reported manganese contents of 15.00mg/ kg in *Brassica napus* and 13.67mg/kg in *Brassica oleracea*. Iron is an essential microelement necessary for haemoglobin formation, normal functioning of the central nervous system, and the oxidation of carbohydrates, proteins, and fats (Adeyeye and Otokiti 1999). Iron content varies from 106.15 to 452.55mg/kg under open field and 149.25 to 323.50mg/kg under polyhouse. The iron content was highest in Long Island Improved genotype under open field which was followed by Franklin F_1 genotype under protected conditions. Baloch et al. (2015) observed iron content 2.83mg/100g in fresh, 27.22mg/100g in dehydrated and 26.22 mg/100g in open sun drying cauliflower. Copper is a vital micronutrient required as a component of various redox enzyme and lignin biosynthetic enzymes. The copper content ranges from (13.25 to 16.10mg/kg) under open field and (10.45 to 18.00mg/kg) under polyhouse. The copper content was highest in (18.00 mg/kg) in Franklin F_1 under protected condition which was followed by Hild's Ideal (16.10mg/kg) under open field. Copper content was 4 and 3mg/kg in *Brassica napus* and *Brassica oleracea* (Lewu and Kambizi 2015). The zinc content ranged from (33.15 to 43.20 mg/kg under open field conditions and under polyhouse conditions(34.95 to 41.05 mg/kg. The Zinc content was highest in Franklin F_1 genotype under open field which was followed by Franklin F_1 under protected conditions. Lewu and Kambizi (2015) observed the zinc content (52.33 mg/kg) in *Brassica napus* was higher than in *Brassica oleracea*(25.33 mg/kg). Baloch et al. (2015) reported zinc content of 1.86 mg/100g in fresh, 17.88 mg/100g in Dehydrated and 17.23mg/100g in open sun drying cauliflower.

Vitamins analysis: The vitamin A content in four genotypes ranged from 0.55mg/kg to 1.99mg/kg in open field while under polyhouse conditions it ranges from 0.83 to 20.9mg/kg (Table 3). Brussels sprouts are ranked as an excellent source of vitamin A due to their high concentration of on account beta carotene content. Within the body beta carotene serves as a precursors, being converted into active vitamin A. The highest vitamin A content (2.09mg/kg) was found in genotype Urja of Brussels sprouts. Anon (2011) reported 35.8µg/100g in Brussels sprouts, 85.9µg/100g in broccoli and 765.8µg/100g in kale. The vitamin C content is ranged from 335.43 to 518.17mg/kg in open field and 378.31 to 582.20mg/kg under polyhouse conditions. The Long Island Improved genotype was reported the highest vitamin C content, measuring 582.20mg/kg. Vitamin C content 90mg/100g in brussels sprouts, 120mg/100g in broccoli, 110mg/100g in kale and 61.5mg/100g in cauliflower were reported in earlier study (Anonymous 2011). Emebu and Anyika (2011) observed that kale contains 23.43mg/100g of vitamin C while Hall (1998) recorded a higher value of 41mg/100g for kale.

Vitamin E content varied from 0.58mg/kg to 80.15mg/kg under open field while under polyhouse conditions it ranges from 1.18 to 1.45mg/kg. In comparison the highest vitamin E content (80.15mg/kg) was observed in Hilds Ideal variety of Brussels sprouts. Emebu and Anyika (2011) reported a vitamin E content of 4.06mg/100g in kale. Vitamin E plays critical biochemical and physiological roles in the body when incorporated into the diet. As a fat-soluble nutrient it is particularly essential in protecting cell membrane from oxidative damage. Acting as an antioxidant vitamin E protects other nutrients and cellular components from oxidative stress induced by reactive agents (Wardlaw and Insel 1995). Brussels sprouts are an excellent source of vitamins K, a nutrient reported to play a crucial role in blood coagulation, bone health and the prevention of cardiovascular diseases (Beulens et al., 2013). Vitamin K content is varied from 0.16 to 0.72mg/kg in open field conditions and 0.10 to 0.25mg/kg under polyhouse conditions. The highest vitamin K content (0.25mg/kg) was observed in Hild's Ideal variety. Vegetables contribute these minerals and vitamins to enhance their availability in daily life. The percentage differences in proximate composition minerals and vitamins content among different genotypes of Brussels sprouts are likely due to variations in factors such as environmental conditions, soil properties, and mineral content in soils etc. Brussels sprouts contribute essential minerals to the diet, and their availability can be influenced by these various factors. These factors

Table 3. Comparison of vitamins in different genotypes of brussels sprouts grown under open and protected environment

Parameters (mg/kg)	Open environment				Protected environment			
	Hild's Ideal	Long Island Improved	Urja	Franklin F_1	Hild's Ideal	Long Island Improved	Urja	Franklin F_1
Vitamin A	ND	ND	0.55	1.99	0.83	ND	2.09	1.27
Vitamin C	424.84	ND	518.17	335.43	378.31	582.20	529.63	391.19
Vitamin E	80.15	ND	4.1	0.58	ND	ND	1.45	1.18
Vitamin K	0.72	0.17	0.16	0.16	0.25	0.19	ND	0.10

ND – Not determine

collectively contribute to the variability in the nutritional content of brussels sprouts, making them a diverse and valuable component of a healthy diet.

CONCLUSION

The determination of proximate composition, mineral and vitamin content of brussels sprouts grown under both environmental conditions will go a long way in providing substantive information on the crop. The highest moisture content in Franklin F_1, ash content in Long Island Improved, crude fibre content in Urja variety and crude fat content in Franklin F_1 under protected conditions. Brussels sprouts are an excellent source of protein having significant content of crude protein in both conditions. Highest total carbohydrates content and total energy content was in Long Island Improved under open field conditions. The Brussels sprouts were discovered to be an excellent source of all required dietary minerals. They provide a variety of important nutrients, including Potassium, Phosphorus, Magnesium, among macro elements and Iron, Manganese, copper and Zinc among microelements. Brussels sprouts despite being a lesser-known vegetable have huge nutritional potential and can be used as a viable substitute for mostly used vegetables. Brussels sprouts are a nutritionally dense vegetable which offers numerous health advantages due to their rich profile of essential nutrients and vitamins. Regular consumption can help to improve digestive health, immune function, bone health, and effective management of blood pressure and blood sugar levels. The presence of antioxidants and phytochemicals further enhances their role in reducing inflammation, protecting against numerous chronic diseases, such as cardiovascular disease as well as certain cancers. Incorporating Brussels sprouts into the diet can be beneficial for individuals of all ages. They can be cooked in a variety of ways, including roasting and steaming, to preserve their nutritional value while improving their flavour. For the best health benefits, consume them as part of a well-balanced diet rich in fruits and vegetables.

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Mechanization in Fruit Harvesting and Potential of Tree Shakers

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***Abstract*:** Fruit picking is the most labour-intensive and time-consuming step of the fruit production pipeline. Conventional methods rely on manual labour for selective picking fruits ready for commercialization and post-harvest processing. Mechanization of fruit harvesting can promote the competence of fruit growers by reducing overall costs, dependence on manual labour, and ensuring timely harvests. This study compiles technological advancements in fruit harvesting mechanization, describing the basic working principles of mechanical harvesting devices and their operational parameters.The main challenges of mechanization include asynchronous fruit maturation, high fruit and tree damage, and diversity in orchard structures. Numerous attempts have been made to address these issues using vibratory harvesters or tree shakers, broadly categorized as air shakers, trunk shakers, limb shakers, and canopy shakers. The specific applications and limitations of these harvesters have been discussed. Additionally, studies exploring abscission chemicals for selective harvesting and customized catching units to minimize fruit damage have been reviewed. This study highlights the potential of mechanical harvesters in reducing labour dependence and costs while emphasizing the need for innovating indigenous harvesting systems.

***Keywords*:** Fruit harvester, Vibratory harvesting, Tree shakers, Canopy shaker, Robotic harvester

In the current period of intensification of highly viable agriculture in India, horticultural crops act as incentives for small and marginal farmers. In India, horticulture accounts for 8.5% of the cropped area and holds 30% of agricultural GDP (Gross Domestic Product). This sector has enough potential to improve the socio-economic status of Indian farmers (Anonymous 2007). Presently, India is one of the largest contributors of fruits and vegetables in the world succeeding China as the largest producers of fruits, India shares a large amount of world's total fruit production of 870 million tonnes (MT) by contributing about 97.35 million tonnes yearly (Anonymous 2018). In India, presently (2018-19), total area under fruit crop cultivation is 86673 ha and the total production is 1850259 MT (Anonymous 2020). A variety of fruits and vegetables are grown in different states of India and are marketed with an advantage in the rest of the country.

The conventional way of harvesting of fruit crops requires high labour input and displays low efficiency in terms of time and overall operational cost. In India, the mechanization of fruit harvesting has been carried out, albeit on a very small scale. The availability of labour in abundance and lack of large-scale fruit farming are the main reasons for the inadequate efforts towards mechanization of fruit harvesting (Hegazy 2013, Kumar and Kalita 2017). However, nowadays, the inclination towards the organized fruit farming is underway, Presently, the fruit cultivation depends on manual labour (Prakash et al*.,* 2023). The labour employed in fruit cultivation is predominantly dedicated to harvesting, which is the most labour-intensive and time-consuming step of the entire production process (Paltrinieri 2015). The natural qualities of fruit products, including shelf-life and perishability, pose a severe time-constraint on the harvesting strategy (Prasad et al*.,* 2018). Harvesting fruits at the optimal stage of maturity is crucial for upholding the postharvest quality as per the satisfaction of the consumer. Hence, attaining optimum quantity of best quality produce in a short time frame is a highly labour-intensive task, which warrants mechanization.

Mechanization plays a vital role in securing the future of fruit growers in developed countries. The use of machinery encourages judicious execution of field operations by reducing the requirement of seasonal labour for small periods of time (Khandetod 2019). Although application of manual harvesting aids/tools reduce overall harvesting costs, their success is surprisingly limited. Further, the conventional system of fruit harvesting from tall tree fruits such as palm, coconut, and mango are prone to serious accidents (Prasad et al*.,* 2018). Hence, development of simple mechanical harvesting devices is necessary since they would allow significant reduction in harvesting labour and help in preventing accidents.

Mechanical harvesting systems are usually designed with the objective of mass extraction of fruits during the season. Such methods involve shaking of limbs, trunks, or canopies of trees. Selection of suitable mechanical harvesters is essential for minimizing the harvest-induced

damage. In most fruit crops, mechanical harvesters are unable to attain the extent of size selection and quality achieved by manual harvesting, and harvesting needs to be followed by a selection procedure for maintaining the quality (Li et al*.,* 2011, Erkan and Dogan 2019). The principal benefit of mechanical harvesting is that the produce may be obtained in short periods of time. However, currently available mechanical systems are unable to replace the fruit selection capability and flexibility of manual pickers. Hence, the sustainability of fruit industries may only be ensured with an optimal adaptation of technological advancements and innovation in mechanical harvesting. Presently, mechanical harvesting technologies mostly utilize canopy shakers (Peterson 1998) and trunk shakers (Torregrosa et al*.,* 2014), and their application is limited to fruits destined for processing. These technologies provide a forced vibration, which is transferred to the fruit resulting in detachment (Castro-Garcia et al*.,* 2019).

In recent years, the horticultural industry has globally supported the innovation of novel fruit harvesting systems. The research in this area is mainly motivated by the need for cost-reduction and enhancement of quality for the consumer. Machine-based harvesting systems provide partial solution for such issues by offering an efficient removal of fruits from the trees and consequently reducing the harvesting cost to about 35-45% of the total production cost (Sanders 2005). Numerous efforts have been made towards mechanized fruit and vegetable harvesting, in various countries (Fig. 1), with significant popularity and remarkable success in diverse crops. Successful mechanization of these operations requires a systems-specific approach, which entails a combined effort of engineers, plant physiologists, food scientists and others.

With this background, this review has been undertaken to describe existing techniques and ongoing researches for harvesting fruits. Various scientific and technical journals have been referred for data collection, with the aim of collating the latest information on the subject to aid the researchers in further innovation.

Principle and Methods for Fruit Harvesting

Among various available methods of fruit harvesting, three are particularly common: manual harvesting of individual fruits by tugging or twisting the fruit pedicel, use of clippers, secateurs, or scissors to detach individual fruits or fruit bunch/vegetables, and use of customized mechanical harvesters (Crivelli 12).

Manual harvesting devices: Two types of manual fruit harvesting devices are available. The first device involves cutting of each fruit independently using sickle or blade, followed by collection. This device consists of a telescopic boom with an adjustable height, and a cutting unit with fixed and movable blades. A wire string is used to shift the movable blade, and fruit is cut and collected in the attached bag (Rajendra 2023). This mechanism is highly suitable in case of delicate fruits, where a risk of damage due to falling is involved, such as in coconut, orange, mango, and papaya. The second device employment positioners for the harvesting of fruits. In this method, ladders are replaced with platforms or moveable worker positioners for harvesting fruit crops such as apples, pears, citrus, dates, papayas, and bananas (Jacob 2008). This device includes a self-propelled machine which enables the positioning of the worker's platform in all the three dimensions. This device permits the use of multilevel picking platforms to enable a continuous movement of the worker while plucking the fruits, which are thereafter placed in a bin or on conveyors. Manual fruit harvesting is highly labour intensive, time consuming, and tedious, and such picking platforms and different man positioners allow a substantial reduction in harvesting cost (Zhang et al., 2022). Overall, these harvesting aids increase the efficiency of labour and help in reducing the harvesting time.

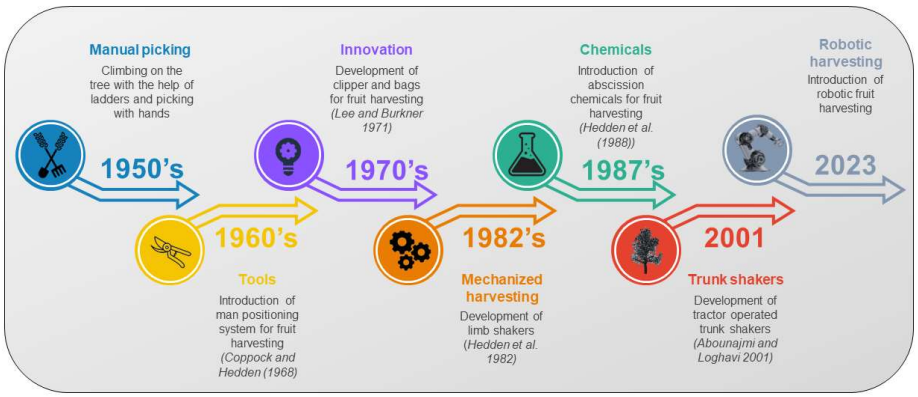

Fig. 1. Timeline of mechanized fruit harvesting

Mechanical harvesting: In general, mechanical harvesting involves application of vibratory or shaking motion on the tree branches (Table 1). Vibrations of suitable frequency and amplitude provide sufficient kinetic energy to the fruits, which are consequently detached from the branches. The vibrations generated from the shaking tool impart a certain force to the branches, leading to the acceleration or deceleration of the branches (Sarkar 2021), which consequently exert a detachment force on the fruits. When the value of the detachment force exceeds that of the attaching force, the fruit is detached from the branch (Liu et al*.,* 2018).

The energy for fruit detachment is provided by the attached trunk or branch shakers. Vibrational motion and the associated forces may be imparted on the tree branch in an efficient and simple manner using inertial vibrators (Chen et al*.,* 2011). The transmission of this vibrational energy to the plant later translates into fruit detachment. To achieve efficient harvesting of fruits with minimal damage, the shaker assembly needs an articulate design and proper operation. The design of a mechanical harvester should not only consider the mechanics of the vibrator and fruit detachment but also aim to minimize damage to the harvested product and the remaining plant structure (Navas et al*.,* 2021). Furthermore, any shear stress on the bark due to slipping of the device should be prevented, since such damage may accumulate over time and lead to the weakening or killing of the tree. Most inertial tree shaker designs employ eccentric rotating masses, and the resulting amplitude depends on the relative mass of the rotating inertial shaker and the mass of the vibrating tree or branch.

The predominant factors influencing the fruit removal efficiency are the biophysical properties of the tree-fruit system and the input vibration parameters. The independent biophysical characteristics of a tree, including the tree architecture (Zhou et al*.,* 2016), branch dimension, and location of fruit, (He et al., 2017) can be experimentally determined; however, accurate characterization of these properties during vibratory harvesting is challenging.

The application of mechanical harvesters for fruit trees is impeded with various problems, such as selection of fruits based on quality and size, possible damage to fruits and trees, and varying orchard designs (Li et al., 2011). To ensure adequate fruit quality and superior aesthetic value for the consumers, harvesting maybe followed by a post-selection process. In general, fruit crops are harvested when maturity is attained. However, several species undergo non-uniform fruit maturation, and therefore require multiple rounds of harvesting with vigilant selection to maximize the yield. Furthermore, fruits are categorized into tree fruits, which include mangoes, apples, and papayas; vine fruits, which include watermelon and muskmelons; and bush fruits, which include raspberries, blueberries, and cranberries. Such diversity in fruits species warrants customization of mechanical harvesters for specific applications, considering the differences in tree habit and orchard structure. Orchard characteristics, such as variety, location, tree age, tree shape, size and spacing, canopy volume, leaf area index, planting density, pruning level, and cultural practices need to be considered for determining the suitability of mechanical harvesters, whereas the type of harvester and catching units needs to be customized optimally to reduce tree and fruit damage. Till date, mechanical vibratory harvesting systems have been successfully employed for olives, oranges (Torregrosa et al*.,* 2009), sweet cherries (Chen et al., 2011, Du et al., 2019) and harvesting apples (Kleine and Karkee 2015).

Based on past studies, four mechanical harvesting approaches have been employed for fruit-picking, viz. air shaking, trunk shaking, limb shaking, and canopy shaking (Table 2). The air shaking technique involves the use of high-speed air blast through large fans to achieve vigorous movement of the canopy. Alternatively, in the trunk shaking approach, the trunk of the tree is grasped shortly above the ground, and the entire tree is vibrated to detach the fruits. Similarly, individual branches are held and shaken in the limb

Table 1. Investigation of factors affecting fruit harvesting efficiency

Fruit	Factor	Findings	References
Apple	Fruit removal efficiency	The optimum frequency was in the range of 8-12 Hz.	Liu et al., 2018
Citrus	The amplitude and Vibration timing	All the fruits were shed at an amplitude of 15Hz when vibrated for 5 seconds	Torregrosa et al., 2009
Mango	Frequency and Vibration timing	Maximum fruit removal was at vibration frequency of 13 to 11 r/s for 4 s.	Parameswarakumar and Gupta 1991
Aonla	Frequency, Amplitude	The limb shaker operated in the range of 50 mm amplitude and 220 cpm frequency for best results.	Kadam 2010
Cherry	Excitation position	As the distance from the excitation location increases, the fruit removal rate in each region increases.	He et al., 2020, Zhou et al., 2014, Du et al., 2010, Junming et al., 2021
	Frequency	The fruit removal rate was higher at 18 Hz frequency.	

shaking approach. Lastly, the canopy shaking approach involves insertion of several flexible horizontal rods into the tree canopy, which are thereafter vibrated to shake the tree canopy through the leaves and branches in contact with the inserted rods. All these approaches achieve fruit removal by vibration of the tree limbs. However, various issues are associated with the idea of fruit harvesting through vibration, such as low productivity, high rate of damage, and low efficiency. Several studies in the recent years have focused on increasing the productivity by enhancing the level of automation of such fruit harvesting systems (Amatya et al., 2016, Colmenero-Martinez et al., 2018).

The quality and applicability of these shaking mechanisms are usually assessed in terms of efficiency of fruit removal and percentage of damaged products. Removal efficiency is calculated as the percentage of fruits that were removed with the harvester. Since the fruits are subjected to various kinds of pressures, such as twisting, bending, and shear forces, the overall removal force and quality are determined in terms of frequency, amplitude, and duration of the vibrations imparted (Loghavi et al., 2010, Zhou et al., 2014). Furthermore, the height at which the fruits are captured affects the fruit quality after impact on the capturing surface (He et al., 24). In addition, the position of attachment of the shaking tool on the tree trunk or branch is crucial for preventing severe damage to the plant.

Mechanical harvesting techniques air shaking: Fruit detachment may be achieved through turbulent air flow applied at a significantly high speed (Homayouni 2021). The fruit harvesting systems based on air shaking employ oscillating air blast machines to maximize the fruit detachment rate by controlling the oscillation rate. Air blast machines based on oscillating air pattern were first introduced in 1961. Thereafter, high-efficiency air blast system capable of generating airflows up to 100 mph (160 km h^{-1}) was developed (Whitney and Patterson 1972). The fruit removal efficiency of these machines were further enhanced with the application of abscission chemicals (Whitney and Wheaton 1987) esp. in crops like olive and citrus (Ferguson 2006). The main limitation of these preliminary machines was the high energy requirement, which required large-sized and heavy engines and fans, rendering the design impractical. Further, air blast harvesters were associated with high fruit damage in apple, thus hampering their popularity (Berlage, 6). Overall, various factors need to be considered to optimize the performance of air blast harvesters, including the tree structure, the fruit size and weight, and the overall fruit load. Based on multiple studies, it may be concluded that air shakers significantly reduce the fruit harvesting time as compared to manual harvesting methods, though with compromised yield. In addition, this method poses a higher risk of damage to the fruits and leaves.

Limb shaking: This method has been predominantly applied for the harvesting of citrus fruits, apricots, prunes, peaches, and sour cherries. Such machines are capable of obtaining large fractions of fruit load owing to the less frequent, but

Table 2. Working principle of different type of fruit harvester

Type of fruit harvester	Working principle	Parameters	Limitation	Fruit type	References
Trunk Shaker	The shaking unit clamps the tree trunk above the bud union and below the first scaffold limbs followed by shaking the tree in straight-line linear direction.	Frequency 18 Hz Acceleration 77 ms^{-2}	• High-shaking frequency • Certain problems damaging trees resulting in fungal attacks	Olives, nuts, citrus, apple, mangoes and pears	Affeldt et al., 1989, Futch and Roka 2005; Sola-Guirado et al., 2014
Limb shaking	By imparting long strokes to limbs at a low frequency or transmission of vibratory forces to the limb.	Amplitude 50 mm Frequency 15 or 20 Hz	• Bark and limb may damage • Even immature fruits can be removed	Citrus fruits, apricots, prunes, peaches and sour cherries	Erkan and Dogan 2019, Refik et al., 2007
Canopy shaker	Fruits are harvested by the vibrating mechanism causing the tines to impact fruit directly or by impacting fruit-bearing branches	Frequency 1.8 or 8.8 Hz, Acceleration 31.4 ms^{-2}	• Less harvesting efficiency • Mechanical damage of fruits (40%)	Citrus, olives	Whitney 1977
Air shaker	High-speed, turbulent airflow for detaching fruits through an oscillating air pattern generated by heavy engines and fans.	airflows up to 160 km/h, energy of 186 to 260 kW	• High fruit damage • Heavy due to the size of the engines and fans	Apple, olive and citrus	Whitney and Patterson 1972; Whitney 1977, Frguson 2006

longer strokes applied on the branches. However, a significant risk of bark and branch damage is associated with this approach, and removal of unripe fruits is possible. The efficiency may be further enhanced by the application of abscission chemicals (Erkan and Dogan 2019). Limb shakers are equipped with a handle to enable remote control, which aids in fruit picking by allowing the workers to access the highest sections of the canopy (Ferguson 2006). A self-propelled version of these shakers was initially applied for the harvesting of 'Valencia' oranges without abscission (Coppock 1971). By optimizing the timing of harvest and application of abscission chemicals, the overall yield loss with mechanical harvesters was significantly reduced in 'Valencia' oranges (Coppock et al., 1971, Sumner et al., 1979). Recently, a handheld limb shaker equipped with a hydraulic motor and slider-crank mechanism was developed for cherry fruit harvesting (Zhou et al., 2014), which was evaluated for the extent of damage and overall efficiency of fruit removal and recommended multiple-point excitation of the branches. Further, a semi-automated fruit harvester including a dual-motor actuator (DMA)mechanism was designed (Kleine and Karkee 2015), capable of generating infinitely variable rhythmic motions. In another recent study (He et al., 2017), the efficiency parameters, including the removal, collection, and recovery efficiencies of a localized shaking and catching mechanism were analysed, wherein the harvesting system was operated on diverse apple varieties.

Overall, the most crucial advantages of limb shakers are providing access to highest sections of the trees and a significant reduction in the harvesting time. However, various problems are also associated with their application, including, low harvesting efficiency in early and mid-harvesting seasons, and undesired detachment of immature fruits at different stages of development, underlining the scope for improvement.

Trunk shaking: Trunk shakers are predominantly applied for the harvesting of deciduous fruits, such as olives, nuts, and citrus fruits. Trunk shaking-based systems provide a sustainable alternative to fruit farmers to survive a competitive market, by reducing the harvesting cost and rendering higher yields. The point of contact of the shaking unit with the tree trunk is immediately above the bud union and below the first scaffold limbs. The design includes a padded, particle-filled clamping pad to linearly vibrate the tree trunk; thereafter the vibration energy is transmitted to the secondary and tertiary branches, leading to fruit detachment. Such straight-line shaking provides an advantage over multi-directional shaking by preventing bark damage. The vibrations are imparted for a duration that varies between 5 and 12 seconds, based on the season and fruit cultivar (Futch and Roka 2005). The operational efficiency is affected by the damping effect of the trunk and branches (Sola-Guirado et al., 2016). To attain optimal harvesting efficiency, the trunk of the fruit tree should be subjected toa high energy vibration. In this regard, trunk shakers provide a time-saving and simpler alternative to limb shakers; however, their applicability is limited to trees thinner than 50 cm in diameter, and to trees without any hanging branches (Tian-Huet al., 2017). A recent report demonstrated the feasible application of the trunk shaker for harvesting citrus fruit, along with the positive effect of the administration of an abscission chemical on fruit detachment (Moreno et al., 2015). The study reported a collection of 70-85% of fruit by the trunk shaker independent of the orchard structure. Moreover, administration of ethephon in higher concentrations displayed a concomitant increase in fruit detachment, with the exception of the 'Clemenules' orchard, thus recommending a judicious use on abscission agents. Overall, trunk shakers are the most popular mechanical harvesters for fruit trees, owing to their superior contribution to mass production. However, trunk shaker operations are usually energy-intensive, which also poses a risk of tree damage. In addition, these harvesters are not suitable for older orchards with distinctive size and shape of the canopy.

Canopy shaking: Fruit harvesters based on canopy shaking accomplish fruit detachment by vibrating the branches at an optimal frequency with the help of multiple rods inserted into the tree canopy. Canopy shakers clamp the secondary branches and generate vertical vibrations, leading to fruit detachment (Sarkar 2021). The mechanism of canopy shaker involves transmission of energy from the inherent shaking rods directly to the canopy branches, depending on the characteristics of the shaking rod. In some cases, a group of shaking rods impact the tree canopy, although not as a single tine. In such systems, the vibrations generated by the shaking mechanism lead to direct impact of the tines on the fruits or the fruit-bearing branches. The most frequently used configuration for such shakers involves the straight shaking rod. The continuous canopy shakers are majorly used, for the harvesting of citrus fruit, and are categorized as self-propelled and tractor-drawn (Erkan and Dogan 2019). The most crucial parameters to assess the function of such harvesters are shaking frequency and stroke. Till date, the contemporary canopy shaking systems have enhanced the fruit harvesting rate to 15 times that achieved by hand-picking (Du et al., 2019).

Over the past decade, numerous modifications of canopy shakers involving straight shaking rods in various configurations have been designed to perform specific

functions for a wide range of fruits. A double-spiked-drum canopy shaker equipped with total twelve whorls was developed by Peterson 1998. Another modified canopy shaker, including 12 sets of free-floating tines radiating out of a vertical axis (Erkan and Dogan 2019) currently serves as the most popular mechanical orange harvester in Florida. Castro-Garcia et al., 2019 developed a canopy shaker for harvesting table olives, consisting of three picking-heads incorporated with straight rubber rods. Furthermore, a continuous canopy shaker, consisting of two self-propelled single drum shakers with 12 whirls of 16 straight tines each, was manufactured by Oxbow International Corporation (Byron, NY, USA)for commercial citrus harvesting (Savary et al., 2010; Spann and Danyluk, 2010. Another experimental canopy rotary drum shaker having multiple straight fiberglass rods were developed (Hong et al., 2012)for harvesting *Jatropha* fruits. A rotary harvester, Korvan 8000,manufactured by Oxbo International (Lynden, Washington, USA), containing two spindles of straight nylon shaking rods, was applied for blueberry with reduced damage. Later, Sola-Guirado et al.,(2014) described the development of canopy shaker with 6 beating drums and 24 straight shaking rods and reported a fruit removal efficiency of 77.3% for olives. In the same year, a citrus canopy shaker was developed and optimized based on a progressive analytical approach devised by Gupta et al. (2016) which allowed minimal damage to the upper canopy. Overall, canopy shakers may be noted as the most efficient category of harvesters, with the capability of continuous harvesting of fruits. Although these harvesters are associated with a large amount of debris and a risk of structural damage to canopies, they in controvertibly display higher fruit removal efficiencies. Moreover, vibrations may be optimized to successfully detach fruits with minimum damage, since branch injuries are more evident at higher amplitudes.

Optimization of shaking parameters required for fruit harvesting: To achieve efficient and continuous harvesting of fruits with shakers to ensure high yield and minimal damage to tree components and fruit products, optimization of harvesting parameters is crucial. The vibration parameters to be provided as input, such as shaking frequency and amplitude, excitation location, and excitation duration, are determined on the basis of the dynamic characteristics of the tree fruit system. These parameters are usually obtained through experimentation and simulation of dynamic models and experimental data. However, most studies in this context have focused on analysing the fruit removal efficiency and extent of damage observed at varying input vibration parameters through field experiments (Leone et al., 2015, Sola-Guirado et al., 2016, He et al., 2017). Various experiments have been conducted to determine the optimal vibration frequency, vibration amplitude, excitation duration (Colmenero-Martinezet al., 2018), excitation location (Zhou et al., 2014), and motor actuator pattern (Kleine and Karkee 2015), leading to the highest fruit removal efficiency and minimum damage. Nevertheless, although the input vibration parameters obtained so far via field experiments are effective, further optimization was required. Hence, a range of amplitude and frequency values were analysed using a laboratory unidirectional vibratory device (Ortiz and Torregrosa 2013), and the effective input vibration parameters according to the target fruit tree were successfully determined through experiments. Since fruit trees display highly variable characteristics, ideally, the input parameters need to be customized for each tree to attain high efficiency; however, such individual in situ experimentation is challenging and unrealistic.

Most of the input vibration parameters remain fixed during tree shaking, except frequency of vibration. Therefore, fruit removal efficiency may be maximized by altering the frequency, while keeping the rest of the parameters as fixed. Notably, when the excitation force is approaching the natural frequencies (Table 3), several branches experience motion at the maximum amplitude. Various approaches and techniques have been used to estimate the natural frequency of tree species, including high-speed digital video and image processing (Geice et al., 2016), pull-release experiments (Kane et al., 2014), and impact excitation experiments (Du et al., 2019). In case of limb shakers, Torregrosa et al. (2009), through his experiments for citrus crop stated that increased stroke length for less number of cycles was considered optimal for sufficient harvesting.

Use of abscission chemicals: Abscission chemicals have been extensively used in field experiments to enhance the efficiency of shaking-based harvesters. The role of various

Table 3. Natural frequencies and amplitudes of different fruit trees

Harvested crop	Frequency (Hz)	Amplitude (mm)
Strawberries	5-15	20-40
Grapes	9-10/10-20	80-140
Oranges	10-15	12-16
Cherries	10-20	15-60
Apple	15-30	8-12
Apricots	15-30	8-12
Almond	15-30	8-12
Peach	15-30	12-16
Olives	20-35	50-70

Source: Ruiz-Altisent et al., 2004, Sarkar 2021

abscission agents has been investigated in reducing the required force for detachment of fruits from the stem (Ebel et al., 2010, Moreno et al., 2015). Administration of abscission chemical increases the difference in the forces required to detach mature and of immature fruits, thus promoting selective fruit harvesting. The application of these chemicals selectively weakens the attachment of mature fruits and decreases the requisite force for their removal. Since the force required to remove the mature fruit from the tree is reduced, tree damage from shaking is also minimized and lesser power is consumed during the harvester operation (Moreno et al., 40). However, biosafety and environment-related concerns associated with the use of abscission agents have now been reported (Chen et al., 2011). These concerns have led to restrictions on the use of these chemicals, limiting their applicability for a wide range of fruit products. With these limitations, the necessity for the development of canopy harvesters capable of functioning independently without abscission chemicals has further increased, along with the importance of further refinement of existing shaker harvesters.

Catching unit: Shaking-based mechanical fruit harvesters are generally equipped with a catching unit. The catching units not only assist in easy collection of fruits, but also protect them from damage. Various materials may be used for the construction of this unit, such as canvas apron, polythene, net material, or foam. Tree shakers enable simultaneous fruit removal from the entire tree, which cause the detached fruits to scatter across the entire catching area. Therefore, the catching units are mostly attached a slow-profile collection surfaces that extend across the area encompassing the outer periphery of the tree. Since the trunk-shaking and catching system is limited to trees with trunk diameter less than 10 inches, tree heights up to 16 ft should be preferred to minimize fruit damage (Futch and Roka 2005). The commonly used catching arrangements include the inverted umbrella wrap-around type and the pained catching units. In 2002, a trunk shaker with a catch frame in the shape of an upside-down umbrella was developed (Vieri 2002). Later, lightweight under-tree nets were fabricated, which were rolled out under the tree on both sides to catch the fruits detached after trunk and limb shaker operations. Furthermore, a catching device consisting of a bounce buffer, a rolling buffer, and a collection area was innovated for apple harvesting, with the objective of reducing the speed and impact of falling fruits (He et al., 2017).

Robotic harvester: In recent times, the field of agriculture has witnessed a paradigm shift in terms of mechanization and automation. However, the number of robotic fruit harvesters developed beyond the research stage remains surprisingly low, none of which have been utilized in open fields. Most robotic harvesters are equipped with manipulators or robotic arms which function over various degrees of freedom, ranging between 3 and 7. Such harvesters have been employed for harvesting of greenhouse-grown strawberries, apples, melons etc. The advanced versions of robotic harvesters also include rotational and prismatic joints to enhance their motility and reach. However, robotic harvesters have low economic sustainability and have witnessed limited success, given their low adjustability to terrain complexity and insufficient speed (Elfferich et al*.,* 2022). Nevertheless, AI and deep-learning based interventions may soon enable them to navigate the orchard types and make adjustment to function over a wide range of tree types.

CONCLUSION

Horticulture is widely recognized as a labour-intensive practice in various regions of the world, including India. Mechanical fruit harvesters provide an alternative solution to manual picking, with a significantly higher rate of harvesting. Such harvesters protect the horticultural process from the influence of the demand and supply dynamics of farm labour, making it more sustainable, and improve the overall profitability and rural landscape. Vibratory harvesters, or tree shakers, are commonly used solutions, and various modifications of basic prototypes have been designed and developed to for specific applications based on fruit type, tree type, or orchard structure. Besides, various interventions, including orchard designing, use of abscission agents, and robotics-related automation have been suggested to encourage the use of tree shakers. The major limitations of mechanized harvesting approaches are the non-uniform ripening of fruit and vegetables, and high fruit and tree damage. Overall, studies suggest an impending need for innovation and customization of indigenous harvesting systems to meet the requirements specific to Indian horticultural ventures.

AUTHOR's CONTRIBUTION

Apoorv Prakash conceptualized the review, performed the literature search, and prepared the initial draft of the manuscript. Anoop Kumar Dixit contributed to the thematic organization, critical analysis, and refinement of content. Dilwar Singh Parihar assisted in literature review, editing and formatting. Arshdeep Singh contributed to manuscript technical validation. Gursahib Singh Manes provided overall supervision, critical revisions, and final approval of the manuscript. All authors read and approved the final version of the manuscript.

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Multi-elemental Profiling of Temi Tea (*Camellia sinensis*) from Sikkim by ICPMS

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Abstract: The experiment was conducted at Sikkim University, Sikkim, India, to quantify the level of multi-elements in different flushes samples of Temi tea (*Camellia sinensis*) which is a renowned organic tea brand grown in the state of Sikkim through ICPMS. In this study the content of copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), cobalt (Co) and aluminium (Al) were analyzed through ICPMS. In Temi tea lead content ranged from 0.18 to 0.26 mg kg^{-1} and cadmium from 0.01 to 0.04 mg kg^{-1} respectively which were below the permissible limit in all the flushes. Other elements were found in good quantity in all the flushes of Temi tea where the copper, zinc, cobalt and aluminium content ranged from 19.69 to 33.74, 0.73 to 1.30, 0.16 to 0.39 and 108.53 to 176.26 mg kg-^{1} respectively and were below the permissible limit.

Keywords: Temi tea, *Camellia sinensis,* multi-elements, ICPMS

Tea is one of the most widely consumed popular beverage in the world (Klepacka et al., 2021) and has become a vital part of human diet (Peng et al., 2024). It has been a staple drink in many countries (Yang et al., 2014) and almost 3 billion people drink tea in more than 160 countries worldwide (Luo et al., 2024). The variety of tea leaves, the conditions of cultivation, harvest and the processing techniques all play a major role in determining these characteristics (Horie et al., 2017, Wu et al., 2018). Of the several steps in the production of tea (leaf handling, withering, rolling, drying, and sorting), fermentation (oxidation) is one of the most crucial (Qu et al., 2019). Black tea is the most popular due to its sensory qualities, but interest in green tea has also been growing for a long time (Yu et al., 2019).

The healthy development of the tea plant and the resulting tea quality as well as the positive health benefits are significantly influenced by the mineral elements and polyphenolic compounds present in the tea leaves (Peng et al., 2022, Tolra et al., 2020). Mineral element accumulation is a concern for both tea producers and consumers (Zhang et al., 2018). The existence of trace elements in tea results from tea plants typically being cultivated in soils that are highly acidic in which trace elements may be more bioavailable for absorption by the roots (Karak and Bhagat 2010). Trace element levels in tea can result in both positive and negative impacts on human health (Atasoy et al., 2019). Minerals, trace elements, and flavonoids are all present in tea and are vital to human health (Thao and Mai 2017).

Tea's high antioxidant activity, which is mostly caused by the presence of phenolic compounds—tannins, catechins and their derivatives is the main factor contributing to beneficial effect on human health (Pinto et al., 2020). The primary active ingredient of tea is catechins which make up 70% of its polyphenols (Yan et al., 2020, Khan et al., 2023). The primary tea polyphenols in black tea are thearubigins and theaflavins (Ke et al., 2021). During tea processing, catechins are oxidized to produce theaflavins (TF), which undergoes enzyme-catalyzed reactions to further transform into thearubigins (TR) (Das et al., 2019). Taste, strength, color, and overall quality of black tea are all greatly impacted by the presence of TF and TR and their ratio (Rahman et al., 2020). Epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate are the primary monomeric forms of catechins found in green tea (Zagula et al., 2017). Polyphenols (catechins and flavonoids), alkaloids (caffeine, theobromine, theophylline, etc.), volatile compounds, polysaccharides, amino acids, lipids, and vitamins are among the biochemical components of tea leaves that exhibit a range of bioactivities (Arachchi et al., 2019). Indian teas are well known worldwide for their unique aroma and taste (Rai 2025). Temi tea is a top-notch organic tea brand which has a huge demand in the international market and is well known for its top-grade tea leaves (Rai and Bag 2024). So far multi elemental study on Temi tea has not been done till date. Considering these facts, the present study focused on the study multi-elemental profiling of Temi tea (*Camellia sinensis*) samples from Sikkim.

MATERIAL AND METHODS

Tea samples of different flush were collected season wise i.e., from Zaid to rabi season (March- November, 2017) from Temi Tea Garden, South Sikkim situated at latitude and longitude of 27.23^{0}N and 88.42^{0}E. The experiment was conducted at Sikkim University, Sikkim, India during

February- March, 2018.

Treatment details: For the multi elemental analysis of the samples, there were five treatments from two types of Temi tea (green tea and orthodox black tea) collected across four season (spring, summer, monsoon and autumn) in completely randomized design with four replications. Multi-elemental analysis was carried out by employing Inductively Coupled Plasma Mass Spectrometry (ICPMS) technique.

Sample digestion and multi elemental profiling: Sample was acid digested in microwave digestion with multi-wave digestion system (Anton Par Multi-wave 3000, India) as per following conditions viz. power- 1200 W; IR - 190 ºC; rate- 0.3 bar sec^{-1}; ramp- 5 minutes; hold – 7 minutes to achieve a clear and colorless solution with the use of Di-acid solution (concentrated Nitric acid and Perchloric acid in the ratio of 9:4). 0.5 g of the leaf sample was taken in the conical flask (100 ml) and 10 ml of Di-acid solution was poured in. The sample mixture was kept on hot plate for digestion and was completed by disappearance of red fumes and appearance of white fumes at the bottom of the conical flask. Little amount of water (5-10 ml) was poured in the conical flask for the dilution of the solution and filtered through filter paper and collected in a 50 ml volumetric flask. The volume was made up to the mark with double distilled water. The clear and colorless solution was stored in the narrow mouth bottle for the multi-elemental analysis in ICPMS. Analysis of the sample was carried out by ICPMS (Perkin Elmer, Nex ION 300 X, USA) with cross flow nebulizer. Standard reference material (peach leaves; NIST, 1547) was used to calibrate the instrument. The ionic composition of the digested sample was examined using a multi-element standards solution.

RESULTS AND DISCUSSION

Copper (Cu): The Cu content in the processed Temi tea samples of different flush ranged from 19.69 to 33.74 mg kg-^{1} (Table 1) which is below the PFA (Prevention of Food Adulteration Act, 1954, India) limit of 150 mg kg-^{1} (Seenivasan et al., 2008) and also below the limits set by the US (150 mg kg^{-1}) (Ning et al., 2011) and Japanese regulations (100 mg kg^{-1}) (Zhong et al., 2016). The highest Cu content was in the green tea (spring flush) while the lowest in black tea (-monsoon flush). Podwika et al. (2018) observed copper concentration in the range of 9.1 to 32.7 mg kg^{-1}. Street et al. (2006**)** reported Cu content in the range of 9-65 mg kg^{-1} and Zhong et al. (2016) in the range 7.73-63.71 mg kg^{-1}which was higher than the present findings. Seenivasan et al. (2008) reported the *Cu* content ranged between 15.9 and 32.2 mg kg^{-1} in South Indian black tea. The present findings also showed higher value than those reported in Iranian tea where the *Cu* content ranged from 24.30 to 32.60 mg kg^{-1} (Abdolmaleki 2016). Herman et al. (2022) in green tea observed Cu content in range of 11.7–18.6 µgg^{-1} in green tea and Koch et al., (2018) in green teas originating from different countries (China, India, Japan, Kenia, and Sri Lanka) in range of 1.34-2.03 mg 100 g^{-1}.

Zinc (Zn): The Zn content in the processed Temi tea samples of different flush ranged from 0.73 to 1.30mg kg^{-1} (Table 1). The highest Zn content was in the black tea (spring flush) while the lowest in black tea (autumn flush). Podwika et al. (2018) reported zinc concentration in the range between 12.6 to 45.5 mg kg^{-1} which are higher than the present findings. Sultana et al., (2023) observed that zinc content of Bangladeshi black tea and green tea in the range 21.58 µg g^{-1} and 5.13 µg g^{-1} respectively and Dawodu et al. (2013) in Nigerian green and black tea 21.17 µg g^{-1} and 30.66 µg g^{-1} respectively. Lahiji et al. (2013) in Iranian tea sample in the range of 38.21 µg g^{-1}, Samali et al. (2012) in Nigerian herbal tea in the range 1.11 to 9.73 µg g^{-1} and Koch et al., (2018) in green teas originating from different countries (China, India, Japan, Kenia, and Sri Lanka) in the range 3.11–4.07 mg 100 g^{-1}.

Cadmium (Cd): The Cd content in the processed Temi tea samples of different flush ranged from 0.01to 0.04 mg kg^{-1} (Table 1). The highest Cd content was in green tea (spring flush) while the lowest in black tea (monsoon flush). Lisia et al. (2015) reported Cd content in Brazilian tea in the range 0.02 to 0.09 mg kg^{-} Rashid et al. (2016) in Bangladeshi in fresh and processed tea reported in the range 0.03-0.13 µg g^{-1} and 0.04-0.16 µg g^{-1} respectively. Zhong et al. (2016) in Chinese tea reported the range of 0.01 to 0.39 mg kg^{-1} and Seenivasan et al. (2008) in the range between 0.05 to 0.38 mg kg^{-1} in South Indian black tea which was higher than the current findings. Zazouli et al. (2010) in Iranian tea was in range 0.09 to 1.92 µg g^{-1}.

Lead (Pb): The Pb content in the Temi tea sample of different flush ranged from 0.18 to 0.26 mg kg^{-1} (Table 1) which is below the PFA limit of 10 mg kg^{-1} (Seenivasan et al., 2008). The highest Pb content was in black tea (autumn flush) while the lowest was in black tea (spring flush). Abdolmaleki (2016) reported Pb content in Iranian black tea samples in the range 0.22 to 0.35 mg kg^{-1} which are higher than the present findings. Lagad et al., (2012) specified individual Pb content of Assam tea, Kangra tea, Darjeeling tea and Munnar tea as 614 ng g^{-1}, 877 ng g^{-1}, 630 ng g^{-1} and 479 ng g^{-1} respectively. Soliman (2016) reported concentration of lead in commercial Egyptian black tea sample was in the range 0.29 to 3.2 µg g^{-1} and Sultana et al. (2023) of Bangladeshi black and green tea in the range 1.39 µg g^{-1} and 3.61 µg g^{-1}respectively. Srividhya et al. (2011) reported in the range 2.31 mg kg^{-1} in tea from South India and Zazouli et al. (2010) in Iranian tea in the range 0.66 to 15.48 µg g^{1} respectively.

Table 1. Levels of multielement in Temi tea samples (mg kg^{-1})

Treatment		Multielement (Mean ± SD)					
		Cu	Zn	Cd	Pb	Co	Al
Spring flush	Green tea	33.74±24.49	1.10±0.80	0.04±0.01	0.25±0.05	0.39±0.05	128.46±103.6
Spring flush	Orthodox black tea	28.15±6.56	1.30±0.39	0.03±0.01	0.18±0.19	0.16±0.06	150.04±37.45
Summer flush	Orthodox black tea	25.50±1.43	1.04±0.09	0.03±0.03	0.24±0.04	0.28±0.05	176.26±20.67
Monsoon flush	Orthodox black tea	19.69±5.55	0.73±0.09	0.01±0.02	0.21±0.08	0.16±0.11	108.53±32.30
Autumn flush	Orthodox black tea	22.32±4.10	0.73±0.12	0.02±0.02	0.26±0.04	0.26±0.06	157.04±30.39

Cobalt (Co): The Co content in the Temi tea samples of different flush ranged from 0.16 to 0.39 mg kg^{-1} (Table 1). The highest Co content was in green tea (spring flush) while the lowest in black tea (spring flush). The findings from the study regarding the cobalt content of tea leaves were in line with previous research where quantities of this element have been observed to be less than 1 mg kg^{-1} in general (Shen and Chen 2008). Lisia et al. (2015) reported the Co content of Brazilian tea in the range 0.19 to 0.38 mg kg^{-1}, Narin et al. (2004) in black tea from Turkey in the range of 7.7 to 30.2 µg g^{-1} and Rohilla et al. (2021) of black tea leaves (tea brew) of Indian market in the range of 71.9-214.3 µg kg^{-1}. Girolametti et al. (2023) observed Co content in black and green teas from tea gardens in six different European countries in the range 0.007 to 0.581 mg kg^{-1} which is higher than the present findings.

Aluminium (Al): The Al content in the Temi tea samples of different flush ranged from 108.53 to 176.26 mg kg^{-1} (Table 1). The highest Al content was in black tea (summer flush) and the lowest in black tea (monsoon flush). The variation of Al content in the present study could be attributed to type of tea, processing technique used and fluctuations in the climatic conditions throughout the growth period. Lahiji et al. (2013) reported Al content of Iranian tea in the range 0.741 ± 0.14 mg g^{-1} and Lagad et al. (2011) specified the Al content of Munnar green and black tea in the range 840 µg g^{-1} and 1367 µg g^{-1} respectively. Ozdemir et al. (2022) reported for black teas produced in Rize, Tabzon and Giresun/Turkey in the range 8177.75 to 15657.72 mg kg^{-1} and Girolametti et al. (2023) Al content in black and green teas in the range 733 mg kg^{-1} to 4865 mg kg^{-1} which is higher than the present findings. A concentration of 40-200 mg kg^{-1} dry weight of aluminium is said to be toxic to crops (White and Brown 2010). Young tea leaves (on an average 380 mg Al kg^{-1} dw) and old tea leaves (on an average 6800 mg Al kg^{-1} dw) both surpass these values (Carr et al., 2003) without exhibiting any signs of toxicity (Pongrac et al., 2020). Al is known to be found in greater amount in tea leaves than in other herbal plants, which hardly ever exhibit concentrations above 1000 mg kg^{-1} (Chizzola 2012).

CONCLUSION

The variation in the elemental content across the different flushes of Temi tea can be attributed to the fluctuations in the climatic conditions throughout the growing period and the different processing method used for making green and black tea. Temi tea had adequate amounts of essential dietary nutrients and was comparable to other premium teas of different origins in terms of elemental concentration.

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Effect of Foliar Application of 2, 4-D, Urea, Zinc Sulphate, Bavistin and Combinations on Nutrient Content of Kinnow Mandarin

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***Abstract*:** The present experiment entitled was conducted at CCS Haryana Agricultural University, Hisar during 2019-20 and 2020-21 to find out the best concentration of foliar application of growth regulators, nutrients and fungicides in improvement of nutrient status of leaves. There were eighteen treatments, *i.e.*, 2,4-D at 10 and 15 ppm, GA_3 at15 and 20 ppm, Urea at 1 and 1.5%, Zinc Sulphate at 0.5and 0.75%, Bavistin 1000 ppm and their combinations on 9-year-old Kinnow. The foliar spray was done two times *i.e.*, first in the last week of May and second in the last week of July. After foliar spray, maximum leaf N (2.51%), Zn (14.97 ppm) and S content (0.22%) was with foliar spray of 2,4-D 15 ppm + Urea 1.5% + Zinc Sulphate 0.75% + Bavistin 1000 ppm, however leaf P, K and Fe were not significantly affected by foliar application of plant growth regulators, nutrients and fungicides.

***Keywords*:** Growth regulators, Nutrients, Fungicides, Kinnow mandarin, Foliar spray

Citrus is one of the most important fruit crops of sub-tropical area belongs to family Rutaceae, sub-family Aurantioideae, tribe Citrae and subtribe Citrinae (Swingle 1943). Among citrus group, Kinnow is a hybrid between King mandarin (*Citrus nobilis* Lour) X Willow Leaf mandarin (*Citrus deliciosa* Tenora), is considered as one of the major citrus fruit crops. It was developed by H.B. Frost in 1915 and released in 1935 in California (USA) and introduced in India in 1958 at the Regional Fruit Research Station, Abohar (Punjab). Kinnow has attained a prime position in North India due to its consumer appeal, good tree vigour, high cropping potential, wider adaptability, more economic return and better performance than other citrus fruits (Kumar et al., 2017). Flower and fruit drop is a major issue faced by citrus growers (Modise et al., 2009) and it has become a limiting factor in citrus production.

Plant growth regulators serve as mobilizers of nutrients from other plant sections to the metabolic sinks that are actively growing (Agusti et al., 2002), which enhance the rapid changes in physiological and biochemical characters and improves crop productivity. Auxin prevents the abscission and facilitated the ovary to remain attached with the shoot and resulted in lower fruit drop (Jat and Kacha 2014). Foliar treatment of GA_3 has been reported to increase production by reducing the per cent fruit drop (Ullah et al., 2014), influence vegetative growth, flowering, fruiting, promotes cell elongation and cell growth and improve fruit retention and fruit quality.

Nitrogen is the most important component for citrus growers and has greater impact on tree growth, appearance and fruit quality than any other element (Obreza 2001). Due to deficiency of nutrients, some healthy orchards are converting into low production with poor quality. The use of growth regulators and chemical fertilizer spray has become an important component of agro-technical procedures for most of the cultivated plants and especially for fruit plants (Prasad et al., 2017). Micronutrients can significantly increase crop yield and quality and improve post-harvest life of produce. They play a significant role in disease resistance and lignin biosynthesis, since they function as enzyme activators (Parmar et al., 2017). In Haryana State citrus quality production is declining due to deficiency of these trace elements caused by soil alkalinity, lower organic matter content and competition from other nutrients (Bhanukar et al., 2018).

Zinc is required for the activity of several enzymes, including dehydrogenases, aldolases, isomerases, transphosphorylases, RNA and DNA polymerases which involved in the synthesis of tryptophan, cell division, maintenance of membrane structure and acts as a regulatory cofactor in protein synthesis (Sharathkumar et al., 2022). The foliar feeding of fruit trees has gained much importance in recent years as fertilizers application through soil are needed in greater amount because some portion leaches down and some does not become available to the plant due to complex chemical reactions (Bisen et al., 2020). Hence, selection of appropriate combination of the plant growth regulators and nutrients is essential to produce high-quality citrus fruits and reduce citrus fruit drop (Kaur et al., 2016). So, keeping in view the above facts and considering the importance of fruit retention to increase the productivity, the present investigation has been framed.

MATERIAL AND METHODS

The present investigation entitled was carried out on nine years old Kinnow mandarin trees at CCS Haryana Agricultural University, Hisar and the chemical analysis was undertaken in Department of Horticulture and Soil Science, CCS HAU, Hisar during the year 2019-20 and 2020-21.

The experiment was laid out in randomized block design with three replications, comprising 18 treatment combinations *i.e.* T_1: 2,4-D 10 ppm; T_2: 2,4-D 15 ppm; T_3: GA_3 15 ppm; T_4: GA_3 20 ppm; T_5: Urea 1%; T_6: Urea 1.5%; T_7: Zinc Sulphate 0.5%; T_8: Zinc Sulphate 0.75%; T_9: Bavistin 1000 ppm; T_{10}: 2,4-D 10 ppm + Urea 1% + Zinc Sulphate 0.5% + Bavistin 1000 ppm; T_{11}: 2,4-D 15 ppm + Urea 1% + Zinc Sulphate 0.5% + Bavistin 1000 ppm; T_{12}: GA_3 15 ppm + Urea 1% + Zinc Sulphate 0.5% + Bavistin 1000 ppm; T_{13}: GA_3 20 ppm + Urea 1% + Zinc Sulphate 0.5% + Bavistin 1000 ppm; T_{14}: 2,4-D 10 ppm + Urea 1.5% + Zinc Sulphate 0.75% + Bavistin 1000 ppm; T_{15}: 2,4-D 15 ppm + Urea 1.5% + Zinc Sulphate 0.75% + Bavistin 1000 ppm; T_{16}: GA_3 15 ppm + Urea 1.5% + Zinc Sulphate 0.75% + Bavistin 1000 ppm; T_{17}: GA_3 20 ppm + Urea 1.5% + Zinc Sulphate 0.75% + Bavistin 1000 ppm; T_{18}: Control. Foliar application was done twice, first in the last week of May and second in the last week of July.

Observation recorded: The content of nitrogen, phosphorus, potash, zinc, iron and sulphur were estimated in the leaf of Kinnow mandarin before and after spray. For determining the leaf nutrients status, five to six months old healthy leaf samples from non-fruiting shoots were collected and washed under running tap water followed by 0.1% HCl and two washings through distilled water. The washed leaf samples were surface dried and then oven dried at 70°C for 48 hours. The dried samples were ground and sieved through muslin cloth for further analysis as per procedure suggested by Chapman (1964). For digestion the 0.5 g quantity of ground leaf sample was taken in 500 ml separate conical flasks and 10 ml diacid mixture (H_2SO_4: $HClO_4$ in 9:1 ratio) was added to each flask. Digestion on a hot plate was carried out as described by Jackson (1967) for the determination of nitrogen, phosphorus and potash. The total volume of aliquot was made to 50 ml.

For the determination of micronutrients *viz*., Fe, Zn and S the 0.5 g of ground leaf sample was taken in 500 ml separate conical flasks and these were digested on a hot plate by adding 15 ml diacid mixture (HNO_3: $HClO_4$ in 4:1 ratio) as per the procedure described by Piper (1966). The total volume of aliquot for microelements was made to 50 ml.

The content of nutrients in the leaf samples was determined by using the following methods:

The nitrogen content and phosphorus content were determined by Nessler's reagent method (Jackson 1973) and by Vanado-molybdo phosphoric acid yellow colour method (Jackson 1973) respectively. The potassium content was determined from the digested extract on flame photometer (Piper 1966). The DTPA extractable Zn and Fe was estimated by using the method of Lindsay and Norvell (1978). The digested leaf samples were analyzed for determining zinc and iron concentration on atomic absorption spectrophotometer and their contents were expressed in ppm. The sulphur content of leaves was determined by turbidity method using spectrophotometer (Chesnin and Yien 1950).

RESULTS AND DISCUSSION

The leaf nitrogen content were significantly affected with different foliar application of growth regulators, nutrients and fungicides and their combinations after foliar spray but leaf potassium and phosphorus content was no significantly affected (Table 1). The leaf N content was maximum (2.51% after foliar spray) with T_{15} (2,4-D 15 ppm + Urea 1.5% + Zinc Sulphate 0.75% + Bavistin 1000 ppm). Leaf N content was significantly affected with various foliar treatments in August during both years of investigation over control. Minimum leaf N content of 1.19% before and 1.23% after foliar spray was in control. Phosphorus and potassium content of leaves was non-significant with foliar application of different concentrations of 2,4-D, GA_3, urea, zinc sulphate and Bavistin. The slight increasing trend in leaf N, P and K content was observed in second year as compared with first year. The increase in nitrogen content with urea might be due to the additional supply of nitrogen to the leaves. Razzaq et al. (2013) also observed that all the trees sprayed with 0.4% zinc sulphate exhibited 1.5-fold higher nitrogen content in leaves in comparison to control. Prasad et al. (2017) concluded that the trees treated with urea, zinc sulphate and 2,4-D had the maximum nitrogen content in leaves of Kinnow mandarin. Bisen et al., (2020) also observed leaf nitrogen was maximum with zinc sulphate (0.6%). Reetika et al. (2020) recorded maximum nitrogen content and level of potassium and phosphorus is not affected with foliar application of urea, K_2SO_4, $ZnSO_4$ in Kinnow mandarin. However, the results of present study are contrary to the findings of Bisen et al. (2020) who reported leaf potassium content was found maximum with zinc sulphate (0.4%).

The zinc and sulphur content in leaf was significantly affected by different foliar applications of various growth regulators, nutrients and fungicides and their combinations after foliar spray (Table 2). Maximum leaf zinc content (14.97 ppm after foliar spray) and sulphur content (0.22% after foliar spray) were in T_{15} (2,4-D 15 ppm + Urea 1.5% + Zinc Sulphate 0.75% + Bavistin 1000 ppm). Minimum leaf zinc content (11.74 ppm before and after foliar spray, sulphur content

Table 1. Effect of foliar application of growth regulators, nutrients and fungicides on leaf nitrogen, phosphorus and potassium content of Kinnow mandarin (%) (Pooled Data of 2019-20 and 2020-21)

Treatments	N content (%)		P content (%)		K content (%)	
	Before foliar spray	After foliar spray	Before foliar spray	After foliar spray	Before foliar spray	After foliar spray
T_1	1.20	2.24	0.15	0.15	1.00	1.02
T_2	1.27	2.31	0.16	0.16	1.01	1.03
T_3	1.20	2.23	0.15	0.15	0.99	1.02
T_4	1.22	2.26	0.14	0.15	1.00	1.02
T_5	1.24	2.31	0.15	0.16	1.01	1.04
T_6	1.30	2.43	0.16	0.18	1.03	1.06
T_7	1.20	2.22	0.14	0.14	0.99	1.01
T_8	1.26	2.30	0.15	0.14	1.00	1.01
T_9	1.26	2.29	0.14	0.14	0.98	1.00
T_{10}	1.30	2.40	0.15	0.17	1.02	1.05
T_{11}	1.28	2.40	0.15	0.17	1.02	1.06
T_{12}	1.22	2.36	0.15	0.16	1.02	1.04
T_{13}	1.30	2.38	0.16	0.17	1.02	1.05
T_{14}	1.32	2.49	0.16	0.19	1.04	1.09
T_{15}	1.31	2.51	0.16	0.19	1.05	1.10
T_{16}	1.31	2.46	0.15	0.18	1.03	1.07
T_{17}	1.31	2.47	0.16	0.18	1.02	1.07
T_{18}	1.19	1.23	0.13	0.13	0.97	0.99
CD (p=0.05)	NS	0.19	NS	NS	NS	NS

Table 2. Effect of foliar application of growth regulators, nutrients and fungicides on leaf zinc (ppm), sulphur (%) and iron content (ppm) of Kinnow mandarin (Pooled Data of 2019-20 and 2020-21)

Treatments	N content (%)		P content (%)		K content (%)	
	Before foliar spray	After foliar spray	Before foliar spray	After foliar spray	Before foliar spray	After foliar spray
T_1	12.20	12.99	0.13	0.14	101.43	103.51
T_2	12.41	13.37	0.14	0.17	101.61	103.87
T_3	12.46	13.10	0.13	0.15	100.90	102.73
T_4	12.60	13.28	0.14	0.16	101.15	103.06
T_5	12.66	13.42	0.14	0.17	101.74	104.20
T_6	12.47	13.73	0.14	0.18	101.85	104.55
T_7	12.62	13.95	0.14	0.18	102.03	104.89
T_8	12.94	14.57	0.15	0.21	102.36	106.66
T_9	12.48	13.23	0.13	0.15	100.22	102.27
T_{10}	13.13	14.50	0.15	0.19	101.71	106.13
T_{11}	12.97	14.52	0.15	0.20	102.40	106.49
T_{12}	12.84	14.21	0.14	0.18	102.23	105.33
T_{13}	12.96	14.33	0.14	0.19	101.91	105.74
T_{14}	13.24	14.93	0.16	0.22	102.97	107.98
T_{15}	13.26	14.97	0.16	0.22	103.56	108.19
T_{16}	13.11	14.70	0.15	0.20	102.43	107.07
T_{17}	13.19	14.76	0.15	0.21	103.00	107.50
T_{18}	11.74	11.74	0.13	0.14	100.18	100.16
CD (p=0.05)	NS	1.17	0.014	0.016	NS	NS

(0.13% before foliar spray and 0.14% after foliar spray) were in control. Iron content was non-significantly affected by all the treatments. All the foliar applications significantly increased the leaf zinc, sulphur and iron content over control after foliar spray. The maximum content of zinc in leaves of Kinnow mandarin might be due to absorption of sprayed micronutrients. Bisen et al. (2020) recorded zinc sulphate (0.6%) increased leaf zinc content. Reetika et al. (2020) observed foliar application of urea, K_2SO_4, $ZnSO_4$, $FeSO_4$ and H_3BO_3 increased leaf zinc content in Kinnow. Rajaiea et al. (2009) observed that foliar application of zinc increased the zinc and iron level in lemon seedlings and Razzaq et al., (2013) recorded that trees sprayed with $ZnSO_4$ (0.6%) showed highest zinc and iron content in leaf of Kinnow mandarin. Khan et al. (2012) observed that combined application of boric acid (0.3%) and $ZnSO_4$ (0.75%) at fruit set stage effectively improved the iron levels in leaves of Feutrell's Early mandarin.

CONCLUSION

The foliar application of 2,4-D 15 ppm + Urea 1.5% + Zinc Sulphate 0.75% + Bavistin 1000 ppm proved most effective in improving leaf N, Zn and S content however P, K and Fein leaf were not significantly affected by foliar application of plant growth regulators, nutrients and fungicides.

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Assessment of Biochemical Components, Mid-Infrared Fingerprints and X-Ray Diffraction Patterns of Ripe and Raw Papaya Fruit Parts

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***Abstract*:** The comparative investigations between ripe and raw papaya fruit pulp, peel and seeds extracts were done including qualitative analysis of bioactive components, morphological analysis and element profile. Alkaloids, polyphenols, tannins and flavonoids were examined in all the papaya samples. Mid-infrared spectroscopy revealed a wide range of functional groups linked with phytocompounds belonging to diverse types of organic chemicals, including alkaloids, polyphenols, amino acids, sulphur, and nitrogen compounds. X-ray diffraction patterns of both ripe and raw pulp extracts showed their amorphous nature. Peel extract of ripe papaya and seed extracts of both ripe and raw papaya showed crystalline nature. Further, the microscopic analysis at 1000X magnification showed reduced tissue integrity in ripe papaya tissues. Additionally, the higher percentage of sodium, phosphorus and potassium were observed in raw papaya pulp. On the contrary, magnesium and sulphur were found in higher concentrations in ripe papaya pulp.

***Keywords*:** Papaya, Fourier transform infrared spectroscopy, Energy dispersive X-ray spectroscopy, X-ray diffraction

The *Carica papaya* is one of the plants with great human health attributes. Although, it is native to Southern Mexico and Central America, it is a popular crop grown all over the world in majorly in tropical and sub-tropical climates. India is the largest producers of papaya followed by Dominican Republic and Mexico. According to Food and Agriculture Organization (FAO), 13.8 million metric tonnes was total annual production of papaya in the world in year 2022 and India alone contributed approx. 38% of the total world production (FAOSTAT, 2024). Papaya fruit contains substantial amounts of vitamins (A, B-complex, C, E and K), minerals, carbohydrates, dietary fiber, lipids and other bioactive phytocomponents. The vitamins-A, C and K provide the fruit excellent antioxidant properties (Nayak et al., 2007, Aravind et al., 2013). Papaya seeds contain phenolic compounds such as glucosinolates, cryptoxanthin carotenoids and isothiocyanates and among fatty acids oleic acid, palmitic acid, linoleic acid are the major constituents (Kermanshai et al., 2001, van Breemen and Pajkovic 2008). The leaves are rich in dietary fiber, polyphenols including, flavonoids, tannins, saponins, anthocyanins and isothiocyanates (Vuong et al., 2013). Besides these key elements, papaya also contains papain a proteolytic enzyme that helps in digestion, treatment of traumatized tissues, sport injuries and allergies (Vij and Prashar 2015). Some studies have also investigated the presence of other industrially important proteolytic enzymes such as chymopapain that also possess anti-viral, antibacterial, antifungal, anti-malarial (Bhat and Surolia 2001, Vij and Prashar 2015). The parts of the whole *Carica papaya* plant such as leaves, roots, fruits, stem and seeds contain important bioactive phytocompounds which may exert various therapeutic effects and can be used as biomedicines. The present study aims to characterize the various parts of papaya fruits including, pulp, peel and seeds of both raw and ripened forms. In addition to the biochemical analysis, microscopic and spectroscopic analysis including mid-infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and energy dispersive X-ray spectroscopy (EDS) were also performed. With a particular emphasis on spectroscopic and microscopic features, the work offers insights on biochemical paradigms that could be useful for future research.

MATERIAL AND METHODS

Fruit collection and extract preparation: Ripe and raw papaya fruits were collected from the local market (26°46´N, 80°55´E), peeled off and sliced into smaller pieces and kept for drying. The dried papaya pulp slices were then ground with mortar and pestle to prepare fine powder and stored. 20 grams of air-dried powders of ripe and raw papaya fruits were soaked in 200 mL of 80% methanol solution (v/v). The prepared suspensions were then homogenized and sonicated (LABMAN Probe Sonicator PRO650) so that most of the phytocompounds can be extracted in the solvent. The prepared suspensions were then kept for 72 hours in shaking incubator for the proper extraction of phytocompounds into the solvent. After 72 hours the prepared suspensions were filtered using grade 1 Whatman qualitative filter papers and

collected the filtrates in fresh vessel. The collected filtrates were then subjected to evaporation in a rotary vacuum evaporator. Then dried extracts were collected and stored at -20°C for further analyses (More and Makola 2020).

Qualitative tests for the presence of bioactive components: The presence of alkaloids, tannins, flavonoids and phenols in the prepared papaya extracts was determined by previously described methods (Ugochukwu et al., 2013, Auwal et al., 2014, Ramya et al., 2015, Pena et al., 2023). The presence of glycosides, steroids and proteins was determined by using previously described methods (Chaudhary et al., 2010).

Evaluation of functional groups through Fourier transform infrared (FT-IR) spectroscopy: Determination of the nature of chemical bonds and probable functional groups that may in turn corelate to the probable phytocompounds/secondary metabolites in PFEs was done by Fourier transform infrared FT-IR spectroscopic analysis. Each powdered extract was independently mixed with potassium bromide (KBr in 1:10 ratio) in mortar and pestle and gently macerated. The mixtures were then palleted by using a hydraulic press. The translucent pallets were used to read and record the absorption spectra in mid infra-red region from wave number 4000-400 cm^{-1} (Sharif et al., 2022) (Model: Nicolet TM 6700, Thermo Fisher Scientific, USA).

Assessment of X-ray diffraction patterns: X-ray diffraction patterns were analysed to determine the amorphous or crystalline nature of the papaya samples. X-ray diffractometer (Bruker, D8 Advance Eco, Germany) was used to analyse the samples. The samples were measured between 10° and 50° angles (2θ) with 0.02° step size. The papaya samples were analysed at a rate of 1 step/second.

Scanning electron microscopic (SEM) examination and energy dispersive X-ray diffraction analysis: For the morphological and elemental analysis, scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were performed respectively. For sample preparation, fresh ripe and raw papaya fruits were washed, peeled off and sliced. The fresh papaya peels and pulps were cut into smaller pieces. For SEM and EDS analysis, the smaller slices of papaya pulp, peel and seeds were dipped into 2.5% glutaraldehyde solution and kept at 4°C for primary fixation, for at ~5 hours. Then to remove the primary fixative, the slices were washed thrice with 0.1M phosphate buffer for 15 minutes each at 4°C. Further the slices were dipped in 1% osmium tetrachloride solution for 2 hours. Again, washed with 0.1M phosphate buffer thrice (15 minutes each). The thin papaya flesh and peel slices were then sequentially dehydrated by dipping in 30, 50, 70, 90 and 95% (v/v) and absolute acetone for 30 minutes in each of the given concentration of acetone (Rizvi et al., 2024). The prepared dehydrated slices were then mounted on black carbon tape on aluminium stubs and kept in vacuum desiccator for further dehydration. The prepared slices were then kept in sputter coater for coating with platinum to make the samples conductive (Auto Fine Coater, JFC 1600, JEOL, Japan). Papaya peels and pulps were visualized by scanning electron microscope (JEOL JSM 6490 LV, Tokyo, Japan) at 1000X magnification.

RESULTS AND DISCUSSION

Biochemical components: Alkaloids, tannins, proteins, flavonoids and phenols were identified in pulp, peels and seeds of both ripe and raw papaya fruit. Glycosides were observed in every part of ripe papaya but in case of raw papaya fruit glycosides were only in peels. Terpenoids were in both ripe and raw papaya fruits except raw papaya peels. Plant based bioactive compounds have great biochemical significance including various medicinalproperties. Alkaloids, tannins, terpenes, flavonoids, phenols and glycosides possess various health benefits and therapeutic effects against critical diseases (Table 1).

Annotation of Fourier transform infrared (FT-IR) peaks: Absorbance of the papaya samples were measured and all potent peaks were labelled (Fig. a-c). In ripe papaya pulp, peel and seeds 11, 11 and 12 peaks were observed in mid-infrared region respectively. In raw papaya pulp, peel and seeds exhibited 10, 9 and 10 significant peaks respectively in mid-infrared region (Table 2). The functional groups were assigned to all detected FT-IR peaks corresponding to the range they fell in (AM 2014, Singh et al. 2022). Most of the spectral peaks of both ripe and rawpapaya samples were falling in the same spectral ranges but with different absorption intensities. The most prominent peaks of both ripe and rawpapaya fruit samples were falling in 3550-3200 cm^{-1} range indicating O-H stretch and in turn the presence of alcohols or phenols. Each of the papaya sample was observed to have at least one spectral peak in 3300-2500 and 3000-2800 range indicating the presence of O-H stretch of carboxylic acid and N-H stretch of amine salts respectively. These ranges might be indicating the presence of amino acids in all the papaya samples. Peaks observed between 2000-1650 and 1750-1735 cm^{-1} corresponded to the tentative presence of aromatic compounds and esters respectively. Between 1550-1500 cm^{-1}, 1543.3 was the only peak in ripe papaya seeds which was corresponding to the presence of nitro compounds in the seeds. In range 1662-1626, 1650-1600 and 1650-1566cm^{-1} alkenes, conjugated alkene and cyclic alkenes were predicted, respectively. Carboxylic acids and alcohols were further predicted in 1440-

1395, 1420-1330 cm^{-1} range. Fluoro compounds with C-F stretch and amines with C-N stretch were predicted in all the samples between 1400-1000 and 1250-1020 cm^{-1} range respectively. The absorbance in the range of 1070-1030 indicated the presence of S=O stretch that might be related to presence of sulphoxides in the samples. Although no peak was observed in ripe papaya seeds in this range, 921.1 was the only peak observed between 950-910 cm^{-1} in ripe papaya pulp that corresponded to O-H bend related to carboxylic acids. The fingerprinting regions of both ripe and rawpapayapulp, peel and seeds indicated the presence of C-Cl stretch (850-550), C=C bend (840-790) and C-Br stretch (690-515) and in turn the presence of halo-compounds and alkenes (Sharif et al., 2022, Singh et al., 2022). The absorption intensity of peaks in the papaya samples were different but most of them were falling in similar spectral ranges indicating the presence of similar kind of functional groups but in different concentrations.

Table 1. Presence of bioactive compounds in ripe and raw papaya pulp, peel and seeds

Bioactive components	Ripe papaya			Raw papaya			Biochemical activities
	Pulp	Peel	Seed	Pulp	Peel	Seed	
Alkaloids	+	+	+	+	+	+	Cardioprotective, anti-inflammatory, anti-cancerous (Heinrich et al., 2021)
Glycosides	+	+	+	-	+	-	Cardioprotective, antithrombotic, analgesic, antidiabetic (Khan et al., 2021)
Steroid	+	+	+	+	-	+	Antiseptic, anti-plasmodial, antimalarial, anti-cancerous (Cox-Georgian et al., 2021)
Tannins	+	+	+	+	+	+	Antimicrobial (Farha et al., 2021)
Flavonoids	+	+	+	+	+	+	Cardioprotective, anti-inflammatory, antiviral, antioxidant, anti-cancerous, neuroprotective (Ullah et al., 2021)
Phenols	+	+	+	+	+	+	Antioxidant, anti-inflammatory, anti-hypertensive (Rana et al., 2021)
Proteins	+	+	+	+	+	+	Building blocks, enzymes

- Absent, + Present

Table 2. Functional group annotation to the FT-IR spectral peaks of ripe and rawpapaya pulp, peel and seeds

Frequencies observed (cm^{-1})						Range	Stretch / bend	Tentative functional groups
Ripe papaya			Raw papaya					
Pulp	Peel	Seeds	Pulp	Peel	Seeds			
3378.2	3418.0	3373.0	3411.3	3396.5	3407.6	3550-3200	O-H stretch	Alcohol / phenol
2927.5	2923.7, 2855.9	2924.9, 2855.4	2924.9	2923.0, 2855.9	2925.3, 2857.2	3300-2500 3000-2800	O-H stretch N-H stretch	Carboxylic acid, Amine salts
1738.0	1734.9	1744.8	1740.1	1735.2	1740.9	2000-1650 1750-1735	C-H bend C=O stretch	Aromatic compound, Esters, δ-lactone
1626.1	1635.7	1648.6	1632.6	1640.8	1633.2	1662-1626 1650-1600 1650-1566	C=C stretch C=C stretch C=C stretch	Alkene, Conjugated alkene, Cyclic alkene
-	-	1543.4	-	-	-	1550-1500	N-O stretch	Nitro compounds
1419.5	1418.3	1455.5, 1379.8	1419.6	1415.7	1412.6	1440-1395 1420-1330 1500-1400	O-H bend O-H bend C-C stretch	Carboxylic acid, Alcohol, Aromatic compound
1253.5 1061.6	1319.2, 1238.5, 1065.3	1237.5, 1157.3, 1104.8	1244.0, 1063.8	1243.2, 1060.8	1244.4, 1060.2	1400-1000 1275-1200 1250-1020 1280-1240 1255-1240	C-F stretch C-O stretch C-N stretch C-O stretch Skeletal vibration	Fluoro compound, alkyl aryl ether, Amine, Epoxides, t-butyl in hydrocarbons
1061.6	1065.3	-	1063.8	1060.8	1060.2	1085-1050 1070-1030	C-O stretch S=O stretch	Primary alcohol, Sulphoxide
921.1	-	-	-	-	-	950-910	O-H bend	Carboxylic acid
820.9 775.4 628.6	777.6, 610.4	614.7	819.4, 775.1, 598.2	608.9	774.3, 605.5	850-550 840-790 690-515	C-Cl stretch C=C bend C-Br stretch	Halo compound, Alkene Halo compound

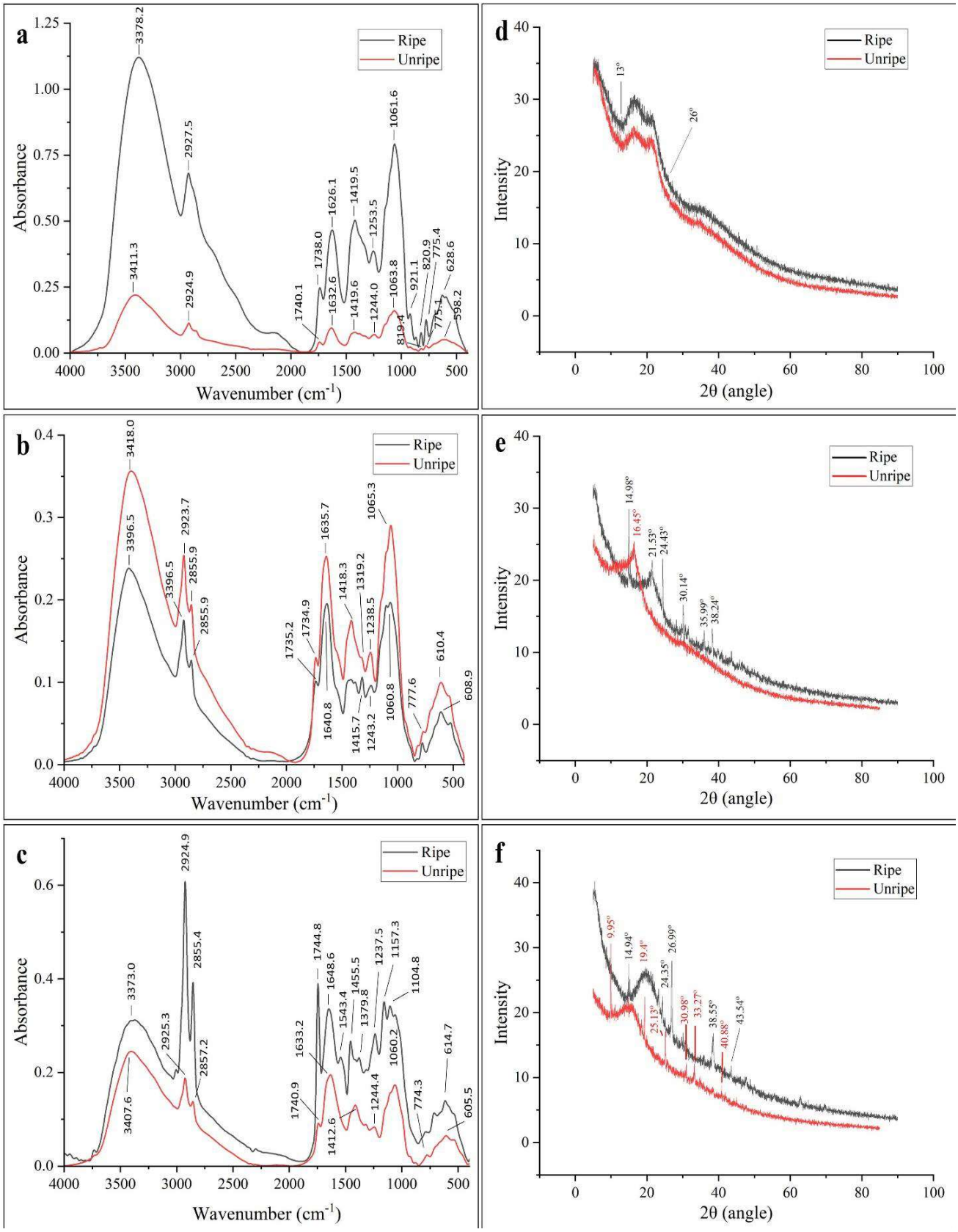

Fig. 1. Comparative analysis of mid-infrared spectra of ripe and raw papayapulp (a), peel (b), seed (c) and X-ray diffractograms of ripe and raw papaya pulp (d), peel (e) and seed (f)

Analysis of XRD: XRD determines the degree of crystallinity of a given sample and identifies its crystalline or amorphous characteristics. The diffractograms of ripe and raw papaya pulp showed “M” type patterns between 13° and 26°(Fig. 1d) with broad peaks indicating the high degree of amorphous structures present in them. Inverted “V” type pattern diffractograms were observed in both ripe and raw papaya peel. The XRD analysis of ripe papaya peel showed significantly sharp peaks at 14.98° and 24.43° depicting its slightly crystalline nature whereas the raw papaya peel extract showed a single broad peak at 16.45° showing its amorphous characteristics (Fig. 1e). “A” type pattern of diffractograms were observed in both ripe and raw papaya seed extracts with various sharp peaks between 10° and 50° (Fig. 1f). In ripe papaya seed extract sharp angles of diffraction were observed at 14.94°, 26.99° and 38.55° whereas in raw papaya seeds extract the diffraction angles were observed at 9.95°, 19.4° and 25.13° indicating the crystalline nature that might be induced by some specific compounds present in both seed samples. The sharp peaks that were observed in the samples might be due to the presence of starch as the crystalline nature of starch varies with the crystalline region and the crystal size (Kim et al., 2005). The crystal size depends on the angle of diffraction and the intensity. If the angle of diffraction is larger while intensity is smaller, the crystal size will be smaller (Singh et al., 2007).

SEM and EDS: Scanning electron micrographs of both ripe and raw papaya epicarp, mesocarp and seeds were obtained at 1000X magnification to analyze the fruit texture, morphological details and the changes during ripening were observed.No significant differences were observed in the image replicates of each sample. With maturity stages, the degradation of tissues becomes more progressive and apparent. Figure 2a shows loosely arranged parenchymatous cells in mesocarp (pulp) which however were compactly arranged of in epicarp (peel) of the ripe papaya (Fig. 2b).Loosely arranged cells having flaky structures were seen in SEM micrographs of ripe papaya seeds (Fig. 2c). Ripe papaya pulp showed less flaky structuresthan the raw one. Distinguishable cellular structures were seen in mesocarp of raw papaya (Fig. 2d), however cellular structures in ripe papaya pulp were not distinguishable and the reduced cellular integrity indicated the reduction in firmness of the pulp. In raw papaya peel, cells were densely arranged showing a high degree of compactness (Fig. 2e). The parenchymatous tissues in ripe papaya became loosely arranged and softened that might be occurred due to the degradation of middle lamella that ismade up of magnesium pectate and hence the level of magnesium was observed to be high in ripe papaya as it was freely available and detected through EDS. However, in raw papaya pulp, cells were densely arranged showing a specific compactness and membrane integrity (de Oliveira and

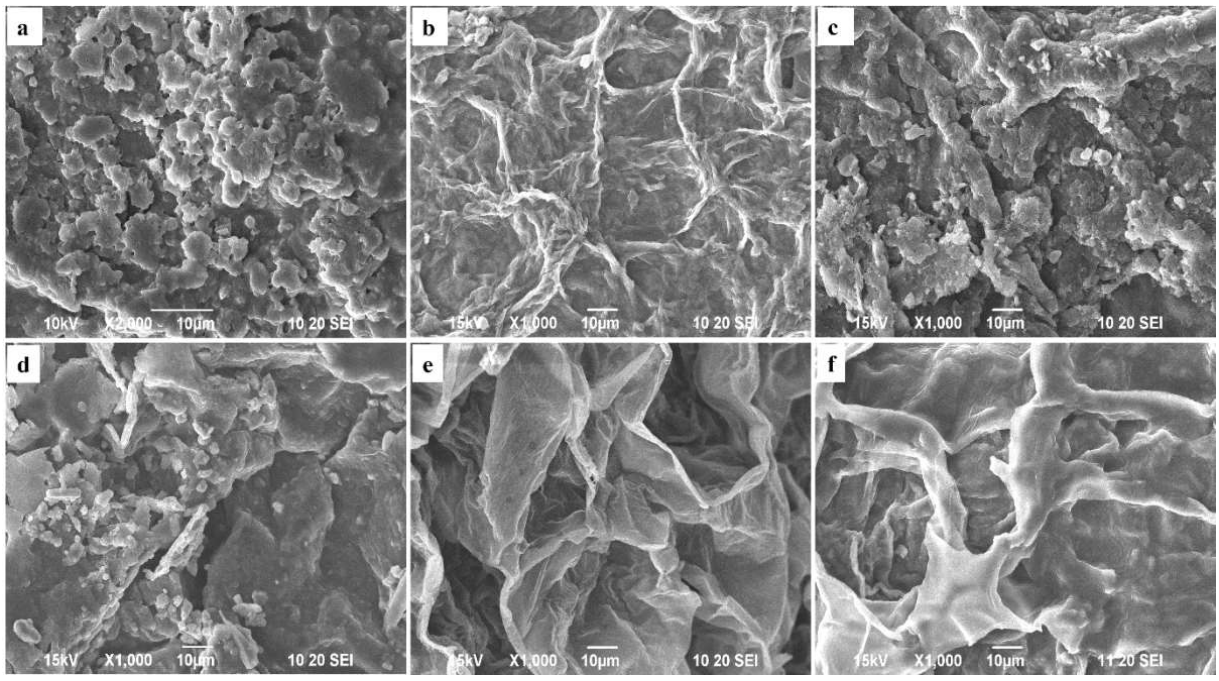

Fig. 2. Scanning electron micrographs of ripe papaya pulp (a), peel (b), seed (c) and raw papaya pulp (d), peel (e) and seed (f)

Vitória 2011, Lara-Abia et al., 2021). The tissues of raw papaya seeds were seemed to be compact with large intercellular spaces (Fig. 2f). The loss of cellular integrity and large intercellular spaces werereported in apple (Quiles et al., 2004) and persimmons (Salvador et al., 2007) during ripening stages.

Energy dispersive X-ray spectroscopy (EDS) is a quantitative analysis which identifies inorganic elements present in a targeted area in the prepared samples in the form of peaks. The peak height increases with the increase in concentration of the elements present. The EDS revealed the richness of ripe papaya pulp in magnesium and sulphur

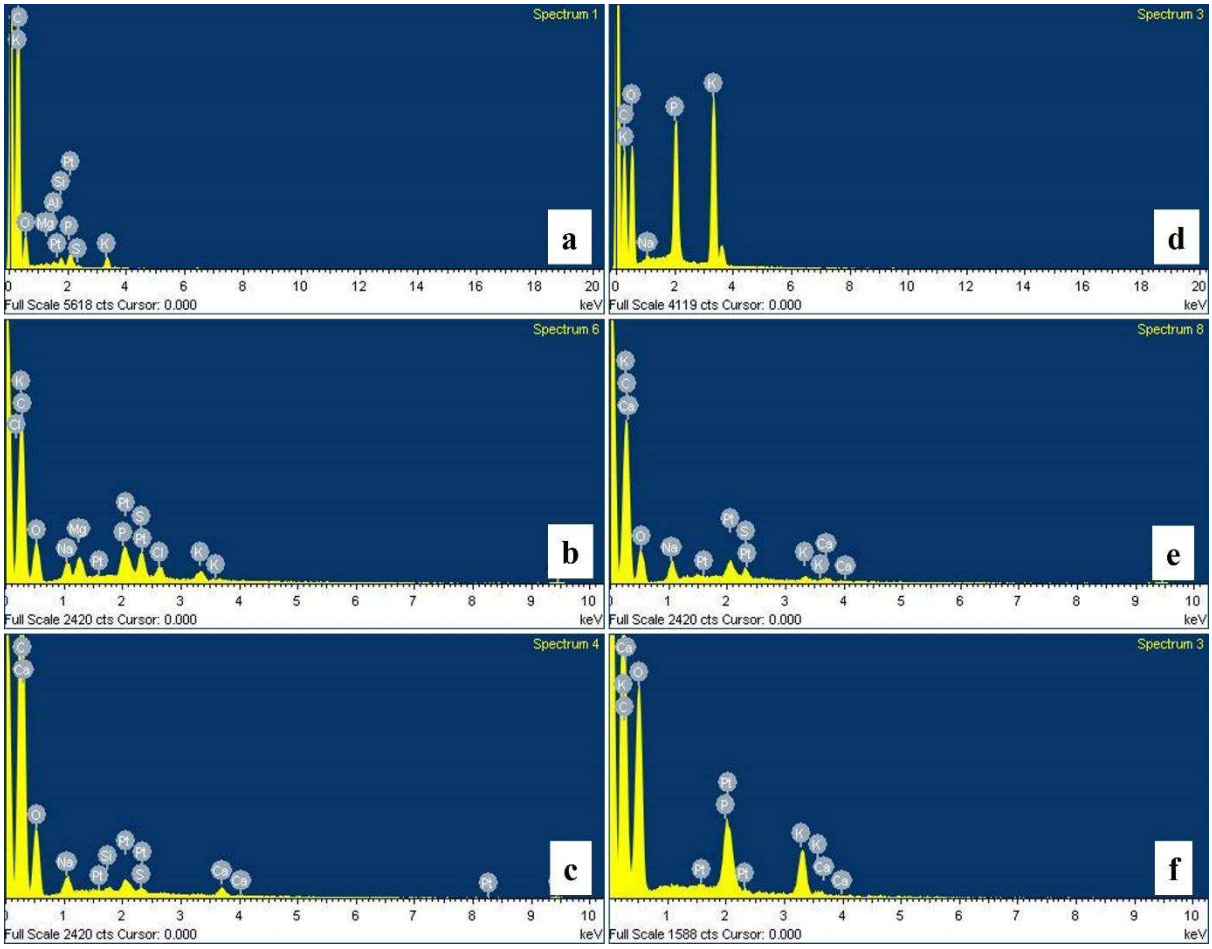

Fig. 3. Energy dispersive X-ray spectra of ripe papaya pulp (a), peel (b), seed (c) and raw papaya pulp (d), peel (e), seed (f)

Table 3. Weight percentage of elements present in ripe and raw papaya fruit pulp, peel and seeds

Elements (weight %)	Ripe papaya			Raw papaya		
	Pulp	Peel	Seed	Pulp	Peel	Seed
Carbon (C)	64.19	58.60	66.17	23.74	56.51	37.19
Oxygen (O)	29.90	27.85	30.31	52.10	32.93	54.14
Sodium (Na)	ND*	1.99	1.25	0.41	3.63	ND*
Phosphorus (P)	0.80	1.80	ND*	9.05	ND*	2.03
Potassium (K)	1.53	1.03	ND*	14.70	0.61	2.76
Magnesium (Mg)	0.27	1.92	ND*	ND*	ND*	ND*
Sulphur (S)	0.19	2.43	0.26	ND*	1.53	ND*
Chloride (Cl)	ND*	1.26	ND*	ND*	ND*	ND*
Calcium (Ca)	ND*	ND*	0.52	ND*	0.65	0.04

ND* is not detected

contents (0.27 and 0.19% respectively) than that of raw papaya (Fig. 3a). Higher level of sulphur in ripe papaya fruit pulp might be due the degradation of various structural proteins via denaturation of their disulphide bonds and thus making it freely available. Although in raw papaya, elements like potassium, phosphorus and sodium (14.70, 9.05, 0.41% respectively of total weight percentage) were present in higher concentrations (Fig. 3d).The EDS spectra of ripe papaya peel (Fig. 3b) showed the presence of potassium andsulphur in higher concentration than the raw papaya peel (Fig. 3e). However, sodium was in higher concentrations in raw papaya. Additionally, phosphorus, magnesium and chlorine were detected in ripe papaya although calcium was not detected which was present in raw papaya peel. Sodium and sulphur were present in ripe papaya seeds (Fig. 3c) whereas these elements were not detected in raw papaya seeds (Fig. 3f). Phosphorus and potassium were in higher concentrations in raw papaya seeds but not detected in ripe papaya seeds. Although calcium was detected in ripe papaya seeds but not in raw papaya seeds. During preparing the samples for SEM-EDS analysis the samples were subjected to platinum coating in the sputter coater and that why the peaks of platinum (Pt) can be seen in the EDS spectra (Table 3).

CONCLUSION

The present study showed the presence of bioactive components in ripe and raw papaya samples. However, in raw papaya pulp and seeds glycosides were not detected. The FT-IR analysis also showed the occurrence of similar kind of functional groups in both ripe and raw papaya samples but in different concentrations. The XRD analysis showed the amorphous nature of pulp extracts whereas the crystalline nature of seeds extracts of both ripe and raw papaya. SEM analysis showed the gradual change in the morphology of pulp, peel and seeds during ripening of the fruit. The elemental profile of the both papaya fruits' pulp, peel and seed revealed that occurrence of various elements in different weight percentage. Further analytical studies can identify the beneficial phytocompounds present in papaya. Morphological studies of the fruit at different ripening stages should be endorsed to understand its mechanism and then to hypothesize strategies to delay the fruit ripening and increase the shelf life of the fruit.

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Minimising Agrochemicals Dependency Through Native Fermented Concoctions: Integrated Nutrient Management Practice for Broccoli Production

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Abstract: Minimising agrochemical dependency to maintain soil fertility and productivity in broccoli (*Brassica oleracea* L. var. *italica* Plenck), an integrated nutrient management approach is a prerequisite. Standardizing doses, including inorganic fertilizers and native fermented concoctions, needs to be established for better results. Application of 90% of the recommended dose of nutrients (RDN) as 112.5 N:67.5 P:46.8 K kg ha^{-1} + cow manure of 20 t ha^{-1} + 5% jeevamrit [10 kg of cow dung, 10 L of cow urine, 2 kg chickpea flour, handful of soil, 2 kg of jaggery (made by boiling, filtering, and condensing sugarcane juice to prepare blocks) in 200 L of water and fermented for 7 days] @ 1.5 Lm^{-2} biweekly and 5% jeevamrit foliar application at a 20-day interval produced tallest plants (58.58 cm), number of leaves per plant (17.86), days to the formation of 50% marketable heads (86.00), number of secondary heads per plant (12.93), weight of the central head per plant (417.55 g), head size (130.88 cm^2), marketable yield per plot (12.61 kg) and per hectare (18.67 Mt) and harvest duration (41.17 days). Black rot incidence was least due to treatment with 100% RDN [(125N: 75P: 52K kg ha^{-1}) + cow manure 20 t ha^{-1}] + Beejamrit (mixed 5 kg cow dung + 5 L cow urine + 250 g lime + handful soil in 20 L water) (20.68 %) used for seed treatment. All native fermented concoctions proved to be beneficial for sustainable vegetable production.

Keywords: Broccoli, Beejamrit, Jeevamrit, Ghana Jeevamrit

The hunger and poverty intensify globally, strategic planning in agriculture is crucial for food security and poverty alleviation. The Pradhan Mantri Dhan Dhanya Yojana, a recent initiative, promotes sustainable farming in 100 low productivity districts to enhance the income of 17 million farmers, with Integrated Nutrient Management (INM) playing key role in supporting their transition towards improved yields and sustainable agricultural practices (Ministry of Finance 2025, The Indian Express 2025).

Historic evidence supports the use of native natural fertilizers and soil amendments for enhancing productivity by securing soil fertility. However, modern agriculture, propelled by scientific advancements and food security concerns, has transitioned to an industrial model reliant on agrochemicals (Verma et al., 2020, Das et al., 2022, Saharan et al., 2023,Goss et al., 2013, Pandian et al., 2024;). While Green Revolution increased production and profitability, it also brought health issues and economic burdens (Rahman 2015, Ameen and Raza 2018, Bjornlund & Bjornlund, 2024, Swastika et al., 2024). This industrial farming model has led to severe social, environmental and economic consequences ranging from health impacts and loss of crop diversity to soil fertility decline and financial strain (Horrigan et al., 2002, Alletto et al., 2022). Despite these challenges, scientific research often influenced by corporate interests continues to promote agrochemicals (Aktar et al., 2009, Lin et al., 2011, Popp et al., 2013, Ncube, 2020, Ahmad et al., 2024). This has increased farmers' reliance on market-driven agricultural inputs under the guise of improving soil and crop health. However, natural farming, which reduces dependence on chemicals and utilizing locally available resources (Jallow et al., 2017, Khan et al., 2021).

In contrast, natural farming is a chemical-free alternative, using farm-based inputs like cow dung, fermented plant concoctions and improved agronomic practices (Laishram et al., 2022, Vashishat et al., 2023). Key components of natural farming include Beejamrit (a seed treatment solution), Jeevamrit (a liquid bio-fertilizer and pest repellent, also available in solid form as Ghanjeevamrit), Achhaddan (live or dead mulching) and Whapsa (soil aeration and moisture management techniques) enhance soil health, yield and disease resistance (Devakumar et al., 2014, Bhadu et al., 2021).

Overuse of chemical fertilizers has degraded soil and increased health risks including nitrate accumulation in crops like broccoli (Webb and Buratini, 2018). Fermented organic concoctions like Jeevamrit (Enhance mineralization, boost microbial activity), Beejamrit (protects against seed borne diseases through antibacterial and antifungal properties) and Ghanjeevamrit (improve soil fertility) offer a sustainable

alternative. These manures enhance soil fertility, crop vigour and organic matter decomposition (Devakumar et al., 2014, Hammad et al., 2019;).

These cost-effective, locally sourced inputs support sustainable agriculture and aim to revive pre-Green Revolution practices prioritizing environment and farmer well-being (Vashishat et al., 2021, Shraddha et al., 2023, Vashishat et al., 2024;). This study evaluates the integrated effects of bio-enhancers and chemical fertilizers on yield and quality of broccoli, contributing to the broader movement toward sustainable and eco-friendly agricultural practices.

MATERIAL AND METHODS

The experiment was conducted at Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India, during winter seasons of 2018-2019 and 2019-2020. The experimental farm lies at 30° 52' north latitude and 77° 11' east longitude. The farm's location is in a sub-humid, sub-temperate, and mid-hill climatic zone. The field experiment was conducted with 3 replications arranged in a randomised complete block design . The two-year field experiment was conducted with ten treatments, T_1- RDN {(125N: 75P: 52K kg ha^{-1}) + FYM 20 t ha^{-1}}; T_2- T_1 + Bj (Seed treatment with Beejamrit); T_3 - 90% RDN + 5% Jeevamrit soil drenching at fortnightly interval (0.5 Lm^{-2}); T_4- 90% RDN + 5% Jeevamrit soil drenching at fortnightly interval (1.0 Lm^{-2}); T_5 - 90% RDN + 5% Jeevamrit soil drenching at fortnightly interval (1.5 Lm^{-2}); T_6- 90% RDN + 5% Jeevamrit soil drenching at fortnightly interval (0.5 Lm^{-2}) + Jeevamrit foliar spray starting at 20 days after planting at 20 days interval; T_7- 90% RDN + 5% Jeevamrit soil drenching at fortnightly interval (1.0 Lm^{-2}) + Jeevamrit foliar spray starting at 20 days after planting at 20 days interval; T_8- 90% RDN + 5% Jeevamrit soil drenching at fortnightly interval (1.5 Lm^{-2}) + Jeevamrit foliar spray starting at 20 days after planting at 20 days interval; T_9 - 90% RDN + Ghana Jeevamrit soil application at 15 and 45 DAP @ 200 $kgha^{-1}$; T_{10}- 90% RDN + Ghana Jeevamrit soil application at 15 and 45 DAP @ 200 $kgha^{-1}$ + Jeevamrit 5 % foliar spray starting at 20 days after planting at 20 days interval.

All the organic preparations were prepared according to the method given by Devvrat (2017). Beejamrit was prepared by mixing cow dung (5 kg), cow urine (5 L), lime (250 g), soil (handful) and water (20 L) and was left out for 24 hours for the fermentation process to carry out (Fig. 1). Twice daily, the mixture was stirred. This mixture was added with the seeds, thoroughly mixed, and then allowed to dry in the shade. For Jeevamrit in a plastic drum, fresh cow manure (10 kg) and cow urine (10 L) were added. Water was combined with jaggary (2 kg), pulse flour (2 kg), and living soil (Handful) to create a final volume of 200 L (Fig. 2). Twice daily, mix all the ingredients in a clockwise motion (morning and evening). The solution was filtered on the fifth day, and the filtrate was prepared for soil drench by diluting it. For soil drenching, Jeevamrit @ 5 per cent (5 L per 100 L of water) was applied at fort nightly interval. The very first application was given from seventh day of sowing and last application implemented fifteen days before harvesting. For foliar application the filtrate was diluted with water and 5 % solution was applied at 20 days interval. Ghana Jeevamrit is the paste prepared from cow dung (100 kg), jaggery (1 kg), gram flour (2 kg), cow urine (1 L) and handful of soil. This paste was covered with a gunny bag for two days, and water was poured on top of it to keep it moist so that appropriate fermentation could occur (Fig. 3). At 15 and 45 days after transplanting, it was formed into a ball about the size of a walnut and applied in the field near the roots of the plant.

Seed of broccoli cv. Palam Samridhi were sown for seedlings. Regular watering, hoeing, and weeding were done from time to time. Approximately 1 month old seedling of broccoli were transplanted on 8 October 2018 and 14 October 2019 with the plot size of 3.0 × 1.8 m and a spacing of 60 × 45 cm. The cv. Palam Samridhi is a green-coloured type

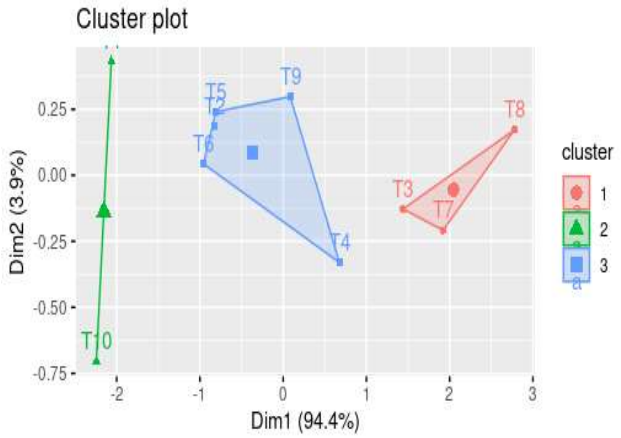

Fig. 1. Plant height cluster plot

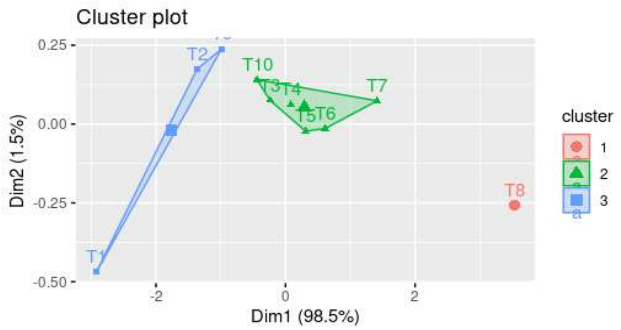

Fig. 2. Number of secondary heads per plant

that matures by 75 to 80 days after transplanting and has a net head weight of 300 to 400 g. Average yield ranges from 15000-20000 kg ha^{-1}. Calculations were done on the basis of treatments with manure, urea, SSP and MOP and desired quantity were incorporated as the basal dose. Urea was applied in split doses; the first at sowing and the remaining half again in 2 splits, first 1 month after sowing and 1 month after the first application. Five plants were randomly selected from each plot. Plant height was recorded at harvest, based on the distance from the ground to the apex of the longest leaf. Days from seedling transplanting to when 50% of plants in plots had marketable heads were determined. For head size polar and equatorial diameters of each head were measured. Numbers of days between the first central head harvest and the last secondary head harvest in each randomly chosen plant per plot was used to calculate harvest duration. Occurrence and severity of black rot, were periodically noted. Black rot disease severity was determined using 10 randomly chosen leaves from 5 plants in each plot that were at various heights (from top to bottom). According to William et al. (1972), an illness rating was performed, and the Mc Kinney (1923) method was used to compute percent disease severity. The scale for disease ratings was 0 to 9 with 0 = no visible signs; 1 = leaf marginal necrosis; 3 = small lesions in a V on the leaf; 5 = a V-shaped lesion covering half of the leaf; 7 = a V-shaped lesion reaching the midrib, and 9 = severe chlorosis and necrosis. Per-cent disease severity was determined by dividing the sum of all disease ratings by total number of ratings multiplied by maximum disease grade.

The cost of cultivation was determined. Multiplying yield by sale rate determined, gross income. The sum of fixed cost per hectare, the risk factor, the management factor, and cost of treatment per hectare was used to compute total cost of cultivation. The whole cost of cultivation was subtracted from gross income to determine net income. Benefit cost ratio was produced by dividing net income by the total cost of cultivation (Sharma et al., 2008).

All the parameters investigated were recorded over the course of two years and subjected to statistical analysis. Pooled treatment means were compared in OPSTAT utilizing the Duncan Multiple Range Test (DMRT) at a significance level of 5 % [Duncan 1955].

RESULTS AND DISCUSSION

Growth parameters: Treatment T_8 (90 % RDN (125N: 75P: 52K kg/ha) + FYM 20 @ t/ha) + 5 % Jeevamrit @ 1.5 L/m^2 at fortnightly interval + 5 % Jeevamrit foliar spray at an interval of 20 days) improved plant height and number of leaves (Table 1). The plant height cluster plot visually groups the data based on height (Fig. 4). Cluster 1 (Red), Cluster 2 (Green) and Cluster 3 (Blue) represent plants with relatively high, low and moderate plant height respectively.

Yield parameters: Minimum days to 50 % maturity of marketable heads was in T_8 whereas maximum days was recorded in T_9 (Table 2). In the first year, 2018-19, T_8 had the fewest days to 50% marketable head, whereas treatment T_1 had the highest. The different trend was seen in 2019–20,

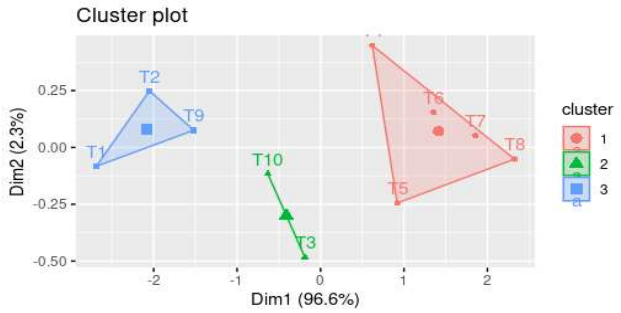

Fig. 3. Weight of central head

Table 1. Effect of native fermented amendments under an INM system on plant height and number of leaves in broccoli

Treatment	Plant height (cm)			Number of leaves		
	2018-19	2019-20	Pooled	2018-19	2019-20	Pooled
T_1	51.00^{f}	51.90^{c}	51.45^{e}	14.25^{c}	14.49^{b}	14.37^{b}
T_2	52.96^{de}	53.52^{bc}	53.24^{de}	15.33^{bc}	15.48^{b}	15.41^{b}
T_3	55.93^{bc}	57.57^{a}	56.62^{abc}	15.37^{bc}	15.38^{b}	15.38^{b}
T_4	55.33^{bc}	55.57^{b}	55.45^{bc}	15.62^{bc}	15.71^{b}	15.67^{b}
T_5	53.08^{de}	53.52b^{c}	53.30^{de}	15.66^{bc}	15.75^{b}	15.71^{b}
T_6	52.33^{ef}	53.78b^{c}	56.06^{bc}	15.77^{bc}	15.84^{b}	15.81^{b}
T_7	56.34^{ab}	58.33^{a}	57.34^{ab}	16.07^{b}	16.10^{ab}	16.09^{b}
T_8	57.76^{a}	59.39^{a}	58.58^{a}	18.13^{a}	17.59^{a}	17.86^{a}
T_9	54.29^{cd}	54.86^{b}	54.57^{cd}	14.78^{bc}	14.63^{b}	14.71^{b}
T_{10}	50.39^{f}	52.03^{c}	51.21^{e}	15.33^{bc}	15.43^{b}	15.38^{b}

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with treatment T_8 having the fewest number of days to emergence of 50% marketable heads, and treatment T_9 having the most days. Number of secondary heads per plant were highest in T_8 while the least was obtained in T_1. Treatment T_8 produced the most secondary heads throughout the 2018-19 growing season, whereas T_1 had least number of secondary heads. Similar pattern was seen in 2019-20. The Cluster plot (Fig. 5) visualises that Cluster 1 (Red) includes plants with a significantly high number of secondary heads, Cluster 2 (Green) represents those with a moderate number and Cluster 3 (Blue) contains plants with a low number of secondary heads.

Maximum weight of the central head was in T_8 and minimum in T_1. Similar pattern was observed in both growing seasons (Table 3). The maximum head size was in T_8. The Cluster plot accurately represents that Cluster 1 (T_5, T_6, T_7, T_8) had a high central head weight, Cluster 2 (T_3, T_{10}) represents those with a moderate central head weight and Cluster 3 (T_1, T_2, T_9) contains plants with a low central head weight (Fig. 6). Minimum head size was in T_1. Both growing seasons saw a continuation of this pattern. Maximum harvest duration was registered in the treatment T_8. Minimum harvest duration was observed in T_1. Similar pattern was followed in both the growing seasons.

Disease resistance: Disease known as black rot, transmitted through both soil and seeds, was seen. There were substantial difference between the treatments over the 2018-19 growing season (Table 4). The treatment T_2, was determined to have the least amount of black rot severity. However, T_1 showed the highest level of black rot severity. The second year, or 2019-20, did not see the occurrence of black rot disease.

Yield and economics: There were substantial disparities between various treatments (Table 5). Pooled analysis of

Table 2. Effect of native fermented amendments under an INM system on days to 50 % marketable heads and number of secondary heads per plant in broccoli

Treatment	Days to 50 per cent marketable heads			Number of secondary heads per plant		
	2018-19	2019-20	Pooled	2018-19	2019-20	Pooled
T_1	92.00^{a}	89.33^{ab}	90.67^{a}	10.93^{d}	10.67^{e}	10.80^{d}
T_2	89.33^{abcd}	87.00^{bc}	88.17^{abc}	11.07^{cd}	11.67^{d}	11.37^{cd}
T_3	90.00^{abcd}	88.33^{ab}	89.17^{abc}	11.33^{bcd}	12.13^{cd}	11.73^{bc}
T_4	89.67^{abcd}	88.00^{abc}	88.83^{abc}	11.40^{bcd}	12.27^{bcd}	11.83^{bc}
T_5	88.33^{bcd}	87.33^{abc}	87.83^{abc}	11.47^{bcd}	12.33^{bcd}	11.90^{bc}
T_6	88.00^{bcd}	86.67^{bc}	87.33^{bc}	11.53^{bc}	12.47^{bc}	12.00^{bc}
T_7	87.67^{cd}	86.33^{bc}	87.00^{bc}	11.67^{b}	12.87^{b}	12.27^{b}
T_8	87.00^{d}	85.00^{c}	86.00^{c}	12.20^{a}	13.67^{a}	12.93^{a}
T_9	91.33^{ab}	90.33^{a}	90.83^{a}	11.13^{bcd}	11.87^{cd}	11.50^{c}
T_{10}	91.00^{abc}	88.67^{ab}	89.83^{ab}	11.27^{bcd}	12.07^{cd}	11.67^{bc}

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Table 3. Effect of native fermented amendments under an INM system on weight of central head, head size and harvest duration in broccoli

Treatment	Weight of central head (g)			Head size (cm^2)			Harvest duration (days)		
	2018-19	2019-20	Pooled	2018-19	2019-20	Pooled	2018-19	2019-20	Pooled
T_1	327.37^{g}	345.21^{g}	336.29^{f}	113.67^{e}	119.87^{g}	116.77^{f}	32.33^{e}	33.67^{d}	33.00^{e}
T_2	339.43^{fg}	354.17^{fg}	346.80^{ef}	115.82^{e}	120.98^{fg}	118.40^{f}	33.00^{e}	35.00^{cd}	34.00^{de}
T_3	371.63^{de}	381.53^{d}	376.58^{d}	121.02^{bcd}	125.13^{cde}	123.08^{cde}	34.67^{bcde}	37.33^{bc}	36.00^{cde}
T_4	380.67^{cd}	399.73^{c}	390.20^{c}	122.04^{abc}	125.90^{bcd}	123.97^{bcd}	36.33^{bcd}	37.67^{bc}	37.00^{bcd}
T_5	388.57^{bc}	400.73^{c}	394.65^{c}	123.66^{abc}	126.38^{bc}	125.02^{bcd}	36.67^{abcd}	38.00^{bc}	37.33^{bc}
T_6	397.60^{b}	406.30^{bc}	401.95^{bc}	124.39^{abc}	126.85^{bc}	125.62^{bc}	37.00^{abc}	38.33^{b}	37.67^{bc}
T_7	401.60^{ab}	418.37^{ab}	409.98^{ab}	124.92^{ab}	128.83^{b}	126.88^{b}	37.67^{ab}	41.67^{a}	39.67^{ab}
T_8	414.60^{a}	420.50^{a}	417.55^{a}	125.96^{a}	135.81^{a}	130.88^{a}	39.67^{a}	42.67^{a}	41.17^{a}
T_9	347.10^{f}	363.57^{ef}	355.33^{e}	117.31^{de}	122.37^{efg}	119.84^{ef}	33.67^{de}	35.67^{bcd}	34.67^{cde}
T_{10}	364.37^{e}	374.84^{de}	369.61^{d}	120.48^{cd}	123.41^{def}	121.95^{de}	34.00^{cde}	36.33^{bcd}	35.17^{cde}

data revealed that maximum marketable yield was in T_8, whereas, minimum marketable yield per plot was in T_1. Similar pattern was observed in 2019-19 and 2019-20. The cluster analysis, based on marketable yield per plot, revealed three distinct groups. Cluster 1 (Blue) had low marketable yield per plot, Cluster 2 (Yellow) consists of samples with medium marketable yield per plot and Cluster 3 (Grey) has high Dim 1 score, indicating high yield (Fig. 7). Figure 8 differentiates yield levels, based on their Dim 1 values, explaining most of the variability. Cluster 1 (Blue), represents medium yield, Cluster 2 (Yellow) high yield and Cluster 3 (Grey) low yield per hectare. Pooled data disclosed that the maximum net income of ₹ 2,45,840 was obtained in t T_8 (90 per cent of recommended dose of fertilizers + 5 per cent drenching with Jeevamrit @ 1.5 litres m^{-2} + 5 per cent foliar spray with Jeevamrit) with net income of ₹ 2,34,757.

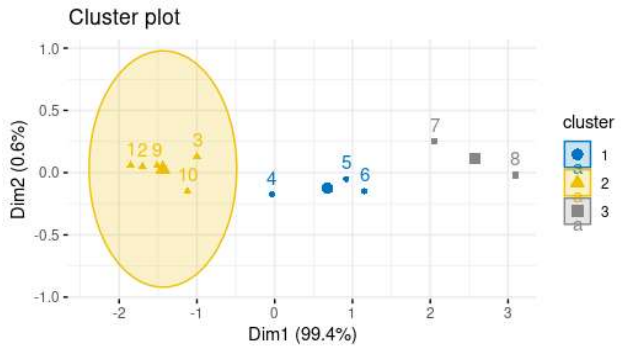

Fig. 4. Marketable yield per plot

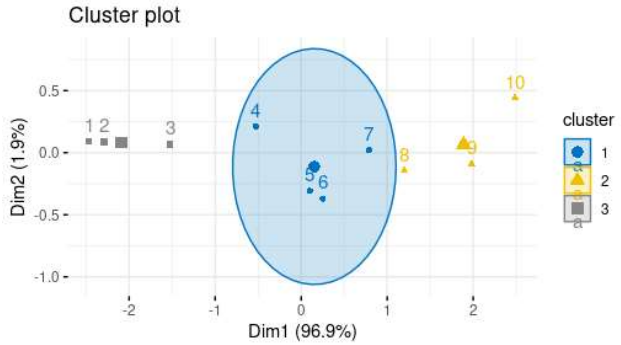

Fig. 5. Marketable yield per hectare

Table 4. Effect of native fermented amendments under an INM system on severity of black rot in broccoli

Treatment	Severity of black rot (%)
T_1	23.31 (4.93) *
T_2	20.68 (4.65)
T_3	21.95 (4.79)
T_4	22.47 (4.84)
T_5	22.63 (4.86)
T_6	22.91 (4.89)
T_7	22.21 (4.81)
T_8	22.04 (4.80)
T_9	22.78 (4.87)
T_{10}	22.61 (4.85)
CD (p=0.05)	NS

The combined application of organic as well as inorganic nutrients enhanced nutrient availability, plant vigour and overall productivity of broccoli. Fermented organic concoctions contributed better soil microbial activity and nutrient assimilation, leading to increased biomass and yield. Plant height and no of leaves per plant are crucial characteristics that connects to other yield-related factors and the morphological framework of plants. While, plant height is primarily governed by genetic factors, but external environmental factors also play a role. The consistent provision of nutrients at regular intervals during all crop growth stages through Jeevamrit, may have enhanced the biological activity of plants, leading to improved cell division, elongation, and increased functional leaf area. These liquid manures could have increased growth and yield-contributing characteristics if combined with chemical fertilisers (Vishwajith and Devakumar 2018). In addition to providing enough food reserves, Jeevamrit treatment increased plant vegetative growth, which may be linked to bioformulations' larger population of benevolent bacteria. These microorganisms contributed to release of accessible nutrients and breakdown of organic materials, which led to increased growth and production (Rathore et al., 2022).

Maximum days to 50% commercial maturity could be because of nitrogen availability in the soil, which delays head initiation. Well rotten manure serves to increase the soil water retention ability and nutrient availability, thereby extending the crop cycle (Negi et al., 2017Kayesh et al., 2019). The increased number of secondary heads per plant observed in the present study can be due to the presence of macro and micro-nutrients as well as additional hormones in fermented liquid organic manures (panchagavya, jeevamrit, beejamrit and ghana jeevamrit) that promote plant growth. Plant vegetative growth and yield-contributing characteristics may benefit from these nutrients [Kumbar and Devakumar 2016, Tiwari et al., 2017].

Yield improvements were evident through enhanced central head weight, head size, and prolonged harvest duration. The presence of beneficial microorganisms in Jeevamrut and other fermented organic amendments likely contributed to improved physical, chemical, and biological

properties of soil. Jeevamrit serves as a source for constant supply of nutrients to plants; some of these nutrients are inorganic and instantly available to plants, while the majority of the other nutrients are released gradually via the mineralization process, resulting in a constant source of nutrients for plants (Gore and Sreenivasa, 2011, Hameedi et al., 2018). Studies support that application of cow urine and farm yard manure, which are the constituents of jeevamrit, beejamrit, and ghana jeevamrit, may have caused an increase in the diameter of sunflower heads due to the beneficial bacteria in the soil which increased fertilizer use efficiency. Enhanced cell permeability causes the respiration process to accelerate, directly increasing the yield-contributing components. Researchers claim that the presence of minerals like Ca, Mg, and Fe causes the biosynthesis of phytin (isoinsitol hexaphosphate), which significantly influence yield [Manjunatha et al., 2009, Negi et al., 2017, Fazeel et al., 2019].

The severity of black rot was not significantly influenced by treatments, yet fermented liquid manures exhibited potential antifungal and antimicrobial properties that may contribute to disease suppression. Similar results in cauliflower were found, that beejamrut was effective in growth promotion and had similar response in damping off management to the chemical thiram [Khatri, 2020]. The absence of favourable conditions for the bacteria to survive may be the cause that disease did not perpetuated in second growing season.

In the rhizosphere, microorganisms frequently multiply, which may have enhanced nitrogen fixation and boosted nutrient availability. As a result, the availability of antibacterial and antifungal agents also rose [Nileema and Sreenivasa et al., 2011, Hameedi et al., 2018,]. With the addition of jeevamrit, microbes may have improved and served as a catalyst. Antioxidants could have been released in the presence of organic matter, which may have been crucial in

Table 5. Effect of native fermented amendments under an INM system on marketable yield per plot and per hectare in broccoli

Treatment	Marketable yield per plot (kg)			Marketable yield per ha (q)		
	2018-19	2019-20	Pooled	2018-19	2019-20	Pooled
T_1	9.95^{e}	10.46^{d}	10.21^{e}	147.47^{e}	154.91^{d}	151.19^{e}
T_2	10.03^{e}	10.53^{d}	10.28^{e}	148.61^{e}	156.02^{d}	152.32^{e}
T_3	10.33^{e}	10.91^{cd}	10.62^{de}	153.07^{e}	161.61^{cd}	157.34^{de}
T_4	10.99^{cd}	11.19^{bc}	11.09^{cd}	162.81^{cd}	165.80^{bc}	164.30^{cd}
T_5	11.39^{bc}	11.72^{b}	11.55^{bc}	168.67^{bc}	173.56^{b}	171.11^{bc}
T_6	11.56^{bc}	11.77^{b}	11.67^{bc}	171.33^{bc}	174.43^{b}	172.88^{bc}
T_7	11.77^{b}	12.44^{a}	12.10^{ab}	174.36^{b}	184.27^{a}	179.32^{ab}
T_8	12.44^{a}	12.77^{a}	12.61^{a}	184.32^{a}	189.21^{a}	186.77^{a}
T_9	10.12^{e}	10.62^{cd}	10.37^{e}	149.86^{e}	157.33^{cd}	153.59^{e}
T_{10}	10.44^{de}	10.69^{cd}	10.56^{de}	154.63^{de}	158.35^{cd}	156.49^{de}

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Table 6. Effect of native fermented amendments under an INM system on economics of broccoli

Treatment code	Yield (q/ha)	Cost of cultivation (₹/ha)	Gross income (₹/ha)	Net income (₹/ha)	B:C Ratio
T_1	151.19	111573	302383	190810	1.71
T_2	152.32	112023	304637	192613	1.72
T_3	157.34	113773	314677	200903	1.77
T_4	164.30	118143	328607	210463	1.78
T_5	171.11	121963	342227	220263	1.81
T_6	172.88	119503	345757	226253	1.89
T_7	179.32	123873	358630	234757	1.90
T_8	186.77	127693	373533	245840	1.93
T_9	153.59	108513	307183	198670	1.83
T_{10}	156.49	114243	312977	198733	1.74
CD (p=0.05)	6.04				0.11

repelling the infections. Jeevamrit possessed a significant amount of microorganisms and growth hormones, which may have enhanced soil biomass and, in turn, the availability and uptake of nutrients, which further increased crop growth and production (Palekar 2006). Jeevamrit is source of advantageous microflora that has the ability to speed up plant growth, including both vegetative and yield aspects [Rathore et al., 2023]. The application of ghana jeevamrit and jeevamrit boosted groundnut yield, which might be ascribed to an improvement in the soil environment, which may have led to an increase in root distribution and nutrient absorption from the soil's bottom layers. By facilitating simple nutrient transfers that are necessary for growth and development, foliar spraying of organic manures increases output (Anusha et al., 2018). The use of organic manures and fermented liquid bioformulations, there were a greater number of fruits per plant in brinjal, which may have been caused by improved nutrition and photosynthesis transfer and subsequent improved plant in growth and yield (Rathore et al., 2022).

Economically, INM with fermented organic concoctions proved advantageous. The highest marketable yield and economic returns were observed with treatments incorporating Jeevamrit and other organic amendments. The combination of beejamrit, panchagavya, and ghana jeevamrit yielded the highest gross income and net worth with the lowest cultivation costs because these organic manures could be produced on the farm itself at no further expense (Chandrakala et al., 2011).

CONCLUSION

The study stresses the potential of minimizing agrochemical dependency by integrating native fermented concoctions with inorganic fertilizers as a sustainable alternative to conventional fertilization in the production of broccoli. The application of 90% RDN with 5% Jeevamrit (drench + foliar spray) significantly improved growth as well as yield-contributing traits while gradually reducing reliance on synthetic fertilizers without compromising the yield. Additionally, seed treatment with Beejamrit successfully mitigated black rot incidence, highlighting the effectiveness of natural bio-inputs in disease suppression. These findings ensure the paradigm shift towards sustainable nutrient management instead of chemical fertilization. By endorsing these native fermented manures, farmers can also contribute to soil preservation and enrichment and support long-term agriculture resilience while preserving high crop yield.

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Effect of Vitamin C on Arsenic Induced Oxidative Stress in Buffalo Erythrocytes in *Vitro*

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***Abstract*:** Exposure to arsenic, a highly toxic trace metalloid causes disturbances in cellular redox status leading to serious health hazards in dairy animals including buffaloes, subsequently affecting human through consumption of milk. Exposure of buffalo erythrocytes to varying concentration of sodium arsenite (0.01-0.5µg for 2hrs.) decreased the activities of superoxide dismutase, catalase, glutathione peroxidase; and increased the malondialdehyde generation and fragility of erythrocytes in a concentration dependent manner compared to the corresponding controls. Vitamin C treatment @10mg/ml to sodium arsenite (0.5µg) challenged buffalo erythrocytes *in vitro* for 2hrs. effectively modulated the oxidative stress as evidenced from the improved membrane lipid peroxidation, erythrocytic fragility and enhanced activities of superoxide dismutase, catalase and glutathione peroxidase compared to the corresponding controls which can be attributed to the antioxidant characteristics of Vitamin C against arsenic. This study demonstrated that acute exposure of sodium arsenite, trivalent arsenic to the buffalo erythrocytes produces oxidative stress through enhanced membrane lipid peroxidation and depletion of intracellular enzymatic antioxidant defense, which was modulated by vitamin C.

Keywords: Arsenic, Oxidative stress, Buffalo erythrocytes, Vitamin C

Arsenic is widely distributed throughout the environment due to its natural existence and anthropogenic application (Dash et al., 2016). It is one of the ten chemicals of major public health concern listed by the World Health Organization. Contamination of arsenic in water, feed, air and soil, in recent times is of global concern (Dash et al., 2016, Mondal et al., 2021). Environmental arsenic exposure is a serious threat to the livestock health, dairy animals including buffaloes as well as to the human health due to its residual effect and transmission via. buffalo milk consumption, making it one health issue. Both acute/ chronic contamination with arsenic may cause oxidative stress (Hu et al., 2020) which leads to serious health hazards in buffaloes (Dash et al., 2016).

Red blood cells (RBC), essential components of the circulatory system, are particularly susceptible to oxidative damage due to their high oxygen carrying capacity (Duan et al., 2017). Biomolecules in RBC are highly vulnerable to oxidative damage (Guidarelli et al., 2017). Redox-imbalance phenomena caused by arsenic exposure are yet to be understood in buffalo erythrocytes, a major hurdle in the development of therapeutic/ preventive strategies (Dash et al., 2016, Qian et al., 2023). Antioxidant therapy is considered as an efficient primary therapy for arsenic exposure (Mukherjee et al., 2017). Synthetic and chemical based antioxidant therapies are having numerous side effects (Williams et al., 2014, Mukherjee et al., 2017). Thus, nutraceutical antioxidant therapy using Vitamin C can be a choice with no side effects for the improvement of erythrocytic oxidative stress status and health of buffaloes exposed to arsenic (Gracia-Rodriguez and Altamirano-Lozano 2017). The current study investigated, if there is involvement of oxidative stress phenomena in buffalo erythrocytes challenged with arsenic *in vitro* and possible antioxidant effects of vitamin C.

MATERIAL AND METHODS

Preparation of erythrocyte suspension: Adult healthy female buffalo (aged 4.5 years) red blood cells were obtained from Innovative Research Inc. biotechnology company, Novi, Michigan, USA. Packed erythrocytes were washed thrice with phosphate-buffered saline (PBS), pH 7.4 and resuspended in PBS. Erythrocytes were counted using hemocytometer and cell number was adjusted to 2×10^6 cells/ml.

Treatment of erythrocytes with sodium arsenite: Erythrocyte suspensions (2×10^6 cells/ml) were incubated with PBS (Control) or sodium arsenite ($NaAsO_2$) (0.01µg to 0.05 µg) for 2 hrs (acute exposure) at 37 °C as follows to study the effects of arsenic exposure on cellular oxidative stress status.

Control group: Erythrocyte suspension incubated with PBS.
Groups T1, T2, T3, T4 and T5: Erythrocyte suspensions incubated with 0.01, 0.02, 0.03, 0.04 and 0.05 µg $NaAsO_2$ for 2 hrs respectively.

[Maximum Permissible Limit of arsenic in drinking water in view of animal health is 0.01µg/ml (WHO)].

Effect of Vitamin C on sodium arsenite treated buffalo erythrocytes: To study the effects of vitamin C, weighed quantity of Vitamin C was dissolved in phosphate-buffered saline (PBS) for the *in vitro* treatment of sodium arsenite challenged buffalo erythrocytes as follows:

Control group: Erythrocyte suspension incubated with PBS.
Groups T1, T2 and T3: Erythrocyte suspensions incubated with $NaAsO_2$ (0.05 µg) and Vit. C (1, 5 and 10mg/ml respectively) for 2 hrs.

Assay of erythrocyte fragility: Erythrocyte fragility was assayed as per the method of Mrugesh et al., (2011). Briefly, after incubation all the erythrocyte tubes were centrifuged at 1500g for 5 minutes. A tube was prepared by adding 2ml distilled water to 2ml RBC suspension to achieve 100% hemolysis. Absorbance of the supernatant was measured by using UV/VIS spectrophotometer (UV-1601, SHIMADZU) at 540nm. Erythrocyte fragility was expressed as the percentage hemolysis.

Estimation of oxidative stress indices: Malondialdehyde, the product of erythrocyte membrane lipid peroxidation was assayed by the method of Placer et al. (1966) and expressed in nmol MDA per mg. Hb. Superoxide dismutase was estimated according to the method of Nishikimi et al. (1972) and results were expressed as U/mg Hb. Catalase assay was carried out as per the method of Aebi (1984). Activity of Catalase was measured using molar extinction coefficient of hydrogen peroxide and expressed as U/mg Hb. Glutathione peroxidase was estimated as per the method of Flohe and Gunzler (1984). Activity of the enzyme was calculated using molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1} and expressed as U/mg Hb. All estimations were carried out using UV/VIS spectrophotometer (UV-1601, SHIMADZU).

Statistical analysis: Data analysis was carried out by statistical package for social sciences (SPSS) software using independent t-tests.

RESULTS AND DISCUSSION

Effect of sodium arsenite (SA) exposure on oxidative stress status in buffalo erythrocytes *in vitro:* Buffalo erythrocytes induced with sodium arsenite (0.01-0.5µg for 2hrs.) *in vitro* exhibited significantly increased formation of malondialdehydes, an indicator of membrane lipid peroxidation in a concentration dependent manner. Maximum generation of malondialdehyde (17.44 fold increase) was observed at 0.5µg sodium arsenite concentration as compared to the control. Followed by the *In vitro* incubation of buffalo lymphocytes with sodium arsenite (0.01-0.5µg for 2hrs.), initially there is an increased response of the enzymes e.g. superoxide dismutase (T1 and T2), catalase (T1), and glutathione peroxidase (T1) as compared to the corresponding controls. However, with increase in dose of sodium arsenite, the activities of superoxide dismutase (T3-T5), catalase (T2-T5) and glutathione peroxidase (T2-T5) significantly decreased in a concentration dependent manner. This was accompanied by an elevation in the fragility of erythrocytes, an indicator of cell membrane damage in a concentration dependent manner (Table 1).

Effect of vitamin C treatment on sodium arsenite induced oxidative stress in buffalo erythrocytes *in vitro:* *In vitro* co-incubation of sodium arsenite (0.5µg for 2hrs.) treated buffalo lymphocytes with vitamin *C* @ 1mg/ml and 5mg/ml for 2hrs did not affect the membrane lipid peroxidation, activities of catalase and glutathione peroxidase and fragility of erythrocytes. But, good improvement in the activity of erythrocytic superoxide dismutase was observed in group T2 (vitamin C treatment is @ 5mg/ml) which continued to improve in group T3 also (vitamin C @ 10mg/ml). Incubation of lymphocytes with sodium arsenite (0.5µg for 2hrs.) and vitamin C (@10mg/ml) simultaneously for 2hrs significantly improved the membrane lipid peroxidation, activities of catalase, glutathione peroxidase and erythrocyte fragility compared to the corresponding controls (Table 2).

This study, for the first time reported that $NaAsO_2$ exposure increased the membrane lipid peroxidation, as evidenced from the formation of malondialdehyde and

Table 1. Erythrocytic fragility and oxidative stress indices (Mean ± SE) in buffalo erythrocytes treated with sodium arsenite *in vitro*.

Parameters	Control	Group T_1	Group T_2	Group T_3	Group T_4	Group T_5
Erythrocyte fragility (%)	0.02 ±0.003	0.64±0.11	36.35±2.01*	49.18±1.16*	60.40±1.64*	86.36±1.42*
Malondialdehyde (nmol/ mg Hb)	1.63±0.14	2.59 ±0.44	8.77±0.51*	13.37±0.71*	20.61±1.12*	28.44±1.04*
Superoxide dismutase (U/mg Hb)	2.94±0.21	3.88±0.51	10.51±0.72*	1.52±0.13*	1.18±0.20*	0.44±0.03*
Catalase (U/mg Hb)	21.82±1.31	25.16±1.12	17.94±1.03*	14.22±1.32*	9.94±1.08*	4.67±0.98*
Glutathione peroxidase (U/mg Hb)	13.83±1.06	14.08±1.30	10.13±1.11*	7.92±0.81*	5.84±0.57*	2.64±0.33*

*Indicates significant ($p<0.05$) difference from control. Data presented above are of eight independent experiments performed in duplicate

Table 2. Effect of Vitamin C on Erythrocytic fragility and oxidative stress indices (Mean ± SE) in sodium arsenite treated buffalo erythrocytes *in vitro*

Parameters	Control	$NaAsO_2$ treatment group (@ 0.05 µg)	$NaAsO_2$ + Vit. C treatment groups		
			Group T_1	Group T_2	Group T_3
Erythrocyte fragility (%)	0.03±0.001	84.71±1.12*	28.24±1.11*	8.16 ±0.44*	0.22±0.02
Malondialdehyde (nmol / mg. Hb	1.88±0.22	27.08±1.28*	20.18±1.06*	9.53±0.77*	2.51±0.24
Superoxide dismutase (U/mg Hb)	3.31±0.18	0.51±0.06*	1.12±0.04*	4.03±0.33	4.19±0.41
Catalase (U/mg Hb)	22.08±1.60	4.44±0.63*	5.96±0.31*	12.99±1.13*	21.10±1.18
Glutathione peroxidase (U/mg Hb)	14.11±1.31	2.93±0.29*	3.40±0.15*	9.51±0.66*	15.31±1.06

*Indicates significant ($p<0.05$) difference from control. Data presented above are of eight independent experiments performed in duplicate

decreased the level of antioxidant enzymes e.g. superoxide dismutase, catalase and glutathione peroxidase in buffalo erythrocytes in vitro creating oxidative stress environment inside the cell. Arsenic compounds causes imbalance in the redox equilibrium of the cell, thereby causing oxidative stress (Gonzalez-Alfonso et al., 2023). $NaAsO_2$ (trivalent arsenic) can generate reactive oxygen species (ROS) through Fenton reaction which produced toxic effects including apoptosis in different types of animal cells (Mondal and Chattopadhyay 2020). In addition to ROS, formation of peroxyl radicals (ROO), superoxide anion radical (O_2^-), singlet oxygen ($^1O_2$), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) was reported in different animals exposed to arsenic (Nithyashree et al., 2023). Lipid peroxidation, protein oxidation, membrane damage, DNA damage and cell death due to arsenite exposure can be attributed to the generation of hydrogen peroxide, followed by OH radicals (Valko et al., 2016). Trivalent arsenic interacts with sulfhydryl group of biomolecules and may cause lipid peroxidation and protein oxidation even at very lower concentrations in very short duration (Nithyashree et al., 2023). The study also reported the enhanced formation of malondialdehyde in $NaAsO_2$ challenged buffalo erythrocytes which increase the fragility of these cells.

Oxidative damage to cellular biomolecules can be modulated by enzymatic and non-enzymatic antioxidants (Ince et al., 2019). Superoxide dismutase and catalase mutually function in the elimination of ROS. ROS inhibit the activities of antioxidant enzymes leading to alterations in cell's intrinsic antioxidant defense causing disturbed antioxidant/pro-oxidant ratio (Nithyashree et al., 2023). In the present study, lowered superoxide dismutase and catalase activity observed in $NaAsO_2$ treated buffalo erythrocytes can be due to enhanced production of ROS during arsenic metabolism or down-regulation of the synthesis of antioxidant enzymes by the toxic arsenite exposure (Mondal and Chattopadhyay 2020). To combat free radical generation, glutathione peroxidase utilizes reduced glutathione during the course of its action (Yadav et al., 2016). Fragility of erythrocytes was increased in $NaAsO_2$ treated groups which can be attributed to increased lipid peroxidation and decreased enzymatic antioxidant status in the buffalo erythrocytes in this study. Elevated membrane lipid peroxidation with depleted antioxidant status in $NaAsO_2$ treated buffalo erythrocytes cause damage to the membrane lipids, oxidative stress and increases the fragility of these cells.

Vitamin C is a powerful antioxidant with superoxide and hydroxyl radical scavenging activity, as well as metal chelating ability (Gracia-Rodriguez and Altamirano-Lozano 2017). However, its therapeutic effect on oxidative stress due to arsenic exposure in buffalo erythrocytes was unclear (Qian et al., 2023). This study observed that $NaAsO_2$ exposure significantly decreased the intracellular enzymatic antioxidant defense in buffalo erythrocytes which was modulated by vitamin C treatment. Vitamin C also modulated the $NaAsO_2$ induced erythrocytic lipid peroxidation in this study. Oxidative stress due to heavy metals can be neutralized by vitamin C which binds to the metal ions, reduce the catalytic activity and resulting ROS production and prevents their harmful effects (Wang et al., 2022). Vitamin C can be easily absorbed and penetrated through aqueous and membrane environment. It can scavenge free radicals due to its electron deficient double bonds which make it highly reactive towards the free radicals (Williams et al. 2014). The therapeutic effect of Vitamin C in this study is attributed to the above antioxidant characteristics, which successfully modulated the oxidative stress induced by arsenite exposure in buffalo erythrocytes.

CONCLUSION

Buffalo erythrocyte damage due to acute arsenic exposure was mediated via. oxidative stress as evidenced

from the generation of malondialdehyde, the lipid peroxidation product and decreased antioxidant enzymes. The oxidative stress phenomena plays vital role in the process of arsenic exposed erythrocyte damage. Vitamin C @ 10mg/ml effectively modulates the redox imbalance status in buffalo erythrocytes and can be used as an ameliorative measure for improving the antioxidant status and health in dairy buffaloes with environmental arsenic exposure.

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Cryptosporidium Genotypes and Subtypes in Sheep in Al-Qadisiyah Province

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***Abstract*:** The accuracy of the sequences was confirmed using *Cryptosporidium* 18SrRNA gene references. The *Cryptosporidium* spp. isolates from sheep faeces included: *C. parvum* (35.29%), *C. hominis* (23.52%), *C. ubiquitum* (11.76%), *C. suis* (11.76%), *C. xiao* (11.76%), and *C. andersoni* (5.88%), with no significant differences . Gene sequence data were recorded in Gene Bank to determine the identity and similarity of *Cryptosporidium* spp. 18SrRNA gene. The 17 samples were compared with global strains in NCBI-Blast. The analysis identified multiple *Cryptosporidium* species, with *C. parvum* being the most prevalent. High sequence homology with global strains, particularly *C. parvum* and *C. hominis*, suggests a significant presence in sheep feces, indicating potential public health risks.

Keywords: Cryptosporidium, Sheep, Genotypes, Subtypes, 18S rRNA gene, GP60 gene

Cryptosporidiosis is a zoonotic parasitic disease caused by *Cryptosporidium*, an intracellular protozoan parasite affecting various vertebrates, including humans. It is particularly significant in domestic ruminants, often causing diarrheal disease in young animals and leading to economic losses (Díaz et al., 2015, Díaz et al., 2018). Transmission occurs through contaminated food and water (Fayer 2000). Diagnosis involves direct, concentration, and staining techniques (Garcia, 2001), while molecular methods like PCR-sequencing provide insights into taxonomy, epidemiology, and genetic diversity. Species classification is based on Gp60 gene analysis, essential for identifying infection sources and assessing public health impact (Stensvold et al., 2015, Leary et al., 2020). The study was conducted on *Cryptosporidium* genotypes and subtypes in sheep in Al-Qadisiyah province

MATERIALS AND METHODS

Sample collection: A total of 200 fecal samples were collected from sheep between October 2020 and March 2021. Samples were obtained from animals of different age groups (<1 and >2 years) and both sexes (155 females and 45 males). The samples were transported in a refrigerated bag to the Parasitology Laboratory, College of Veterinary Medicine, University of Al-Qadisiyah, for further analysis.

Microscopic examination of Cryptosporidium oocysts: The presence of Cryptosporidium oocysts was determined using the Modified Ziehl-Neelsen Staining (MZNS) technique. Each fecal sample was examined through direct smear slides and flotation methods using a NaCl solution.

DNA extraction and molecular analysis: DNA was extracted from all 200 samples using a commercial DNA extraction kit (Add Bio, Korea) following the manufacturer's instructions. The extracted DNA was stored at -20°C until further use in PCR analysis.

Nested PCR: Nested PCR (N-PCR) was used for Cryptosporidium spp. diagnosis based on the 18S rRNA gene. The outer and inner primers used were as follows:

Outer primers:
Forward: 5'-TTCTAGAGCTAATACATGCG-3'
Reverse: 5'-CCCATTTCCTTCGAAACAGGA-3'
Inner primers:
Forward: 5'-GGAAGGGTTGTATTTATTAGATAAAG-3'
Reverse: 5'-AAGGAGTAAGGAACAACCTCCA-3'

For *Cryptosporidium parvum* subtyping, an N-PCR approach was used targeting the GP60 gene with the following primers:

Outer primers:
Forward: 5'-ATAGTCTCCGCTGTATTC-3'
Reverse: 5'-GGAAGGAACGATGTATCT-3'
Inner primers:
Forward: 5'-TCCGCTGTATTCTCAGCC-3'
Reverse: 5'-GCAGAGGAACCAGCATC-3'

For *Cryptosporidium hominis* subtyping, the following primers were used:

Outer primers:
Forward: 5'-TTACTCTCCGTTATAGTCTCC-3'
Reverse: 5'-GGAAGGAACGATGTATCTGA-3'
Inner primers:
Forward: 5'-TCCGCTGTATTCTCAGCC-3'
Reverse: 5'-GCAGAGGAACCAGCATC-3'

Thermal cycling conditions were performed using a thermal cycler (BioRad, USA) and Taq DNA polymerase (AddBio,

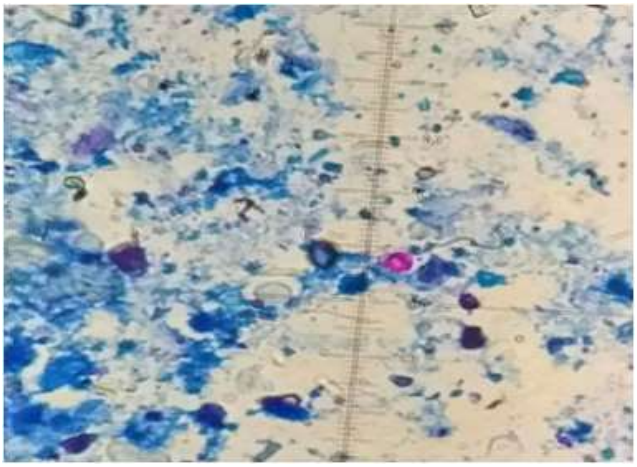

Fig. 1. *Cryptosporidium* spp. oocysts stained with M.Z.N.S at 100x magnification)

Korea). The conditions for the first round included:

Initial denaturation at 95°C for 10 minutes
Followed by 39 cycles of:
95°C for 30 seconds
55°C for 30 seconds
72°C for 60 seconds
Final extension at 72°C for 5 minutes

The second round was performed under similar conditions as the first.

Sequencing: All positive samples were analyzed using molecular methods, and 17 pure DNA samples were sequenced targeting the 18S rRNA gene for genotype identification. The GP60 gene was amplified for *C. parvum* and *C. hominis* subtyping. Ten isolates were sequenced, including six *C. parvum* and four *C. hominis*. The N-PCR

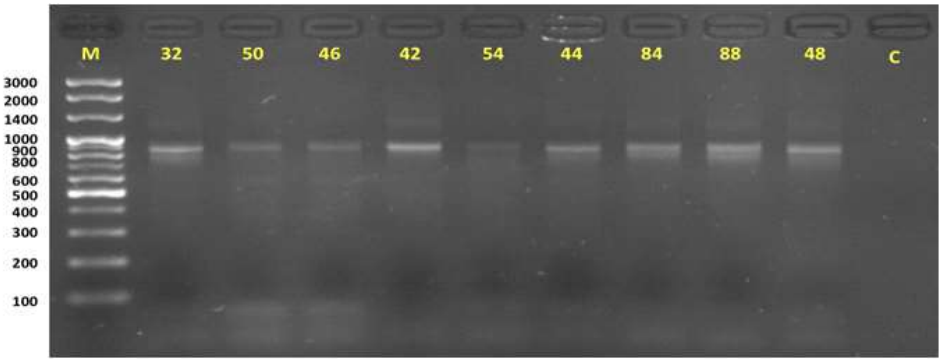

Fig. 2. *Cryptosporidium. spp.* infection is detected by Nested-PCR, targeting 18S ribosomal RNA gene

Table 1. NCBI-BLAST homology sequence identity of *Cryptosporidium* spp. isolates

Accession No.	*Cryptosporidium* spp.	Gen Bank Accession No.	Country	Identity (%)
MW947215	*C. parvum*	AF112570	USA	99.88
MW947216	*C. parvum*	AF112570	USA	99.88
MW947217	*C. parvum*	AH006572	USA	98.24
MW947218	*C. parvum*	KT151531	Iraq	98.12
MW947219	*C. parvum*	KT151531	Iraq	98.24
MW947220	*C. parvum*	AF112570	USA	100
MW947221	*C. hominis*	DQ286403	Chile	100
MW947222	*C. hominis*	AB369994	Saudi Arabia	100
MW947223	*C. hominis*	KF146220	Brazil	99.63
MW947224	*C. hominis*	MK990042	China	100
MW947225	*C. ubiquitum*	MN833282	China	100
MW947226	*C. ubiquitum*	KT027446	USA	99.14
MW947227	*C. suis*	GQ227705	China	99.04
MW947228	*C. suis*	GQ227705	China	98.92
MW947229	*C. xiao*	KM199756	China	99.75
MW947230	*C. xiao*	FJ896050	USA	94.44
MW947231	*C. andersoni*	JN400881	India	100

Table 2. *C. parvum* subtypingwere compared with other NCBI-Blast deposited global strains

Identity (100%)	Country	Gen Bank accession number	Subtype	Accession number
99.87	Spain	KY49903	IIdA17G1a	MW984360
100.00	Spain	KT764969	IIdA17G1	MW984361
99.87	China	KT964799	IIdA17G1a	MW984362
99.62	United Kingdom	GU21438	IId A17G1	MW984363
99.87	Netherlands	MH79636	IIdA17G1a	MW984364
99.61	United Kingdom	HQ14900	IIdA17G1a	MW984365

Table 3. Subtyping of *C. hominis*as compared with other NCBI-Blast deposited global strains

Identity (100%)	Country	GenBank Accession Number	Subtype	Accession number
100.00	China	FJ153239	IbA21G2	MW984366
99.64	China	FJ707313	IbA21G2	MW984367
96.69	Spain	MK105902	IbA21G2	MW984368
99.12	Germany	KM539016	IbA21G2	MW984369

products were sent to Macrogen Co., Korea for direct sequencing, and species and subtypes were identified using BLAST search in the GenBank database.

RESULTS AND DISCUSSION

Diagnostic characterization of *Cryptosporidium* spp.: *Cryptosporidium* spp. oocysts were identified as oval or spherical in shape, appearing dark pink or red on a blue background using Modified Ziehl-Neelsen Stain (M.Z.N.S). The microscopic examination showed that 61 out of 200 samples (30.5%) were positive for *Cryptosporidium* infection. **Molecular examination:** The sequence accuracy was confirmed using *Cryptosporidium* 18SrRNA gene references. The breakdown of *Cryptosporidium*spp. isolates was maximum in *C. parvum* (35.29%) followed by *C. hominis* (23.52%), *C. ubiquitum* (11.76%), *C. suis* (11.76%), *C. xiao* (11.76%) and *C. andersoni* (5.88%). No significant differences were observed at the p-value level of 0.05. Gene sequence data were recorded in the Gene Bank for further comparison

The sequence analysis revealed a variety of *Cryptosporidium* species, with C. parvum being the most prevalent. Sequence homology with other global strains confirmed high identity percentages across the isolates, especially with *C. parvum* and *C. hominis* strains. The results suggest a significant presence of these species in the sheep faeces, which may have implications for public health.

CONCLUSION

This study confirmed the diversity of *Cryptosporidium* species in sheep faeces, with high genetic homology to global strains. Future studies should focus on exploring the epidemiological implications of these findings.

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Quantification of Nitrogen Savings by Efficient Azotobacter Isolate in Tomato (*Lycopersicon esculentum* MILL) Cultivation and Evaluation of Biocontrol Efficacy

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Abstract: A pot experiment was conducted to study the selection of effective *Azotobacter* isolates for tomato (*Lycopersicon esculentum* Mill) in terms of nitrogen accumulation, total N uptake, and antifungal activity of *Azotobacter* isolates. The experiment was carried out during 2022-2023 at the Department of Agricultural Microbiology, College of Agriculture, Raipur, C.G. Tomato seedlings were transplanted on 28 October 2022. An interaction study with *Fusarium* was conducted to determine the nitrogen-fixing efficiency of *Azotobacter* isolates, as well as the fruit and shoot nitrogen content, fruit and shoot nitrogen uptake, and total nitrogen uptake at harvest. The application of the local *Azotobacter* isolate AZOT-B-32 resulted in the highest values for nitrogen accumulation in tomato, total nitrogen uptake, and the antifungal activity of *Azotobacter* isolates.

Keywords: *Azotobacter* isolates, Nitrogen accumulation, *Fusarium* interaction, Antifungal activity

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop due to its high nutritional content, low environmental requirements, ease of cultivation, and high yield and quality. Tomatoes are grown worldwide on approximately 5.1 million hectares and global tomato production is estimated at 182.8 million metric tons annually. The average global productivity of tomatoes is about 35.8 tons per hectare FAO (Food and Agriculture Organization 2023). In India, tomatoes are cultivated on approximately 0.9 million hectares with production of t 20.3 million metric tons annually. The average productivity in India is about 22.5 tons per hectare (Ministry of Agriculture and Farmers' Welfare, Government of India, 2023). In Chhattisgarh, tomatoes are cultivated on approximately 45,000 hectares. The state produces about 1.2 million metric tons of tomatoes annually and average productivity is 26.7 tons per hectare (Department of Horticulture, Government of Chhattisgarh, 2023).

The large number of aerobic and anaerobic bacteria have been identified as free-living nitrogen fixers, with their nitrogen-fixing potential ranging from 2 mg to 25 mg per gram of carbon source utilized. Among these potential nitrogen fixers, *Azotobacter* is key bacteria that fixes nitrogen in non-legumes. *Azotobacter* is a heterotrophic, free-living nitrogen-fixing bacterium commonly found in alkaline and neutral soils. *Azotobacter chroococcum* is the most widely occurring species in the arable soils of India. In addition to ability to fix atmospheric nitrogen, it can also synthesize growth-promoting substances such as auxins, gibberellins, and, to some extent, vitamins. Many strains of *Azotobacter* also exhibit fungicidal properties against certain species of fungi. Its population is relatively low in uncultivated lands, but the presence of organic matter in the soil promotes its multiplication and nitrogen-fixing capacity. The low population density is also due to extreme environmental conditions, including high air temperatures of up to 48°C, soil surface temperatures exceeding 60°C, and low humidity (3-4%) during the prolonged summer season (Bali et al., 2022, Patel et al., 2023). These conditions result in the loss of organic matter and a reduction in the population of beneficial microbes (Chhattisgarh State Agricultural Department 2023).

Broad-spectrum microbes such as *Rhizobium*, *Azospirillum*, *Azotobacter*, phosphorus-solubilizing bacteria (PSB), vesicular-arbuscular mycorrhiza (VAM), and blue-green algae (BGA) are incorporated as active ingredients in biofertilizers and are commercially available on the market (Seenivasagan and Babalola 2021). Some *Azotobacter* isolates influence the synthesis of secondary metabolites and phytohormones, which play a role in tomato yield and quality. These phytohormones affect metabolic pathways (such as amino acids, vitamins, phospholipids, and fatty acids) and induce ethylene synthesis, which, in turn, promotes carbohydrate production and translocation in fruit. Auxins, cytokinins, and gibberellins promote plant growth, biomass accumulation, and agricultural yield (Hindersah et al., 2020, Fusco et al., 2022). *Azotobacter* spp. are sensitive

to acidic pH, high salinity, and temperatures above 35°C, which results in a low population density in the soils of Chhattisgarh. The soils in Chhattisgarh are low to medium in available nitrogen, making nitrogen one of the most limiting plant nutrients. Given the rising prices of chemical fertilizers, coupled with the increasing demand for these inputs and the depletion of soil fertility, there is a need to develop effective bioinoculants of *Azotobacter* for tomato cultivation. The current studies were conducted to develop location-specific *Azotobacter* isolates for tomatoes in Chhattisgarh.

MATERIAL AND METHODS

The experiments were conducted in at Department of Agricultural Microbiology. College of Agriculture, Raipur, Chhattisgarh, India during 2022-23. The experimental farm is located at 21°16 N latitude and 81°36 E longitude with an altitude of 298.56 meters above mean sea level. The climate of the area ranges from dry-sub humid to semi-arid. It is located at 21°16' N latitude and 81°36' E longitude, with maximum temperatures rising up to 46°C during the summer. The mean annual rainfall is 1200-1300 mm, with about 85% of it received from the third week of June to mid-September.

The recommended fertilizer for tomato is 120 kg/ha of nitrogen (N), 60 kg/ha of phosphorus pentoxide (P_2O_5), and 80 kg/ha of potassium oxide (K_2O), respectively. The cultivar used in the study was C.V. Pusa Rubi, procured from the Agricultural College Seed Storage Lab, Raipur, Chhattisgarh, India. There were eleven treatments, complete in a randomized block design : T_1 (AZOT-B-35+100:60:80 NPK), T_2 (AZOT-B-32+100:60:80 NPK), T_3 (AZOT-B-18+100:60:80 NPK), T_4 (AZOT-B-39+100:60:80 NPK), T_5 (AZOT-B-123+100:60:80 NPK), T_6 (AZOT-B-33+100:60:80 NPK), T_7 (AZOT-B-109+100:60:80 NPK), T_8 (IARI ,S.C.+100:60:80 NPK), T_9 (control-I+120:60:80 NPK), T_{10} (control-II+115:60:80 NPK) and T_{11} (control-III+100:60:80 NPK). Forty local *Azotobacter* isolates and *standard Azotobacter* IARI isolate (standard check) were collected from Microbial Culture Bank of Department of Agricultural Microbiology, CoA, Raipur. During this experiment, seven top performing isolates were compared with the same standard check and three uninoculated control contained 100:60:80, 115:60:80 and 120:60:80 kg N, P_2O_5 and K_2O, respectively.

RESULTS AND DISCUSSION

Nitrogen fixing efficiency of *Azotobacter* isolates: The range of nitrogen fixed in the N-free Jensen's liquid medium varied from 2.35 to 13.45 mg N/g of sucrose (0.0047% to 0.0269% N) after seven days of incubation (Table 1). Three local *Azotobacter* isolates: AZOT-B-33, 32 and 18 were at par with standard check (*Azotobacter* IARI isolate). Among all isolates, isolate AZOT-B-33 fixed the maximum quantity of nitrogen in the medium, i.e., 13.45 mg N/g of sucrose (0.0269% N), followed by isolate No. 32 after seven days of incubation. The standard check released13.10 mg N/gm sucrose(0.0262 % N). *Azotobacter* strains have been evaluated for their nitrogen-fixing efficiency and tolerance to abiotic stressors, which has implications for enhancing sustainable agricultural practices (Singh et al., 2021). The nitrogen-fixing bacteria, *Azotobacter* contribute significantly to soil fertility and plant nutrition (Singh and Yadav 2021, Patel et al., 2022, Kumar et al., 2023).

Nitrogen content in fruit: The inoculation of tomato seedlings with local *Azotobacter* isolates and standard check with 100:60:80 kg of NPK significantly increased the N–content in tomato fruit over control C-III (100:60:80 NPK) (Table 2, 4). Maximum nitrogen content in fruit was 1.95 % in T_6 (AZOT-B-33+100:60:80 NPK) followed by T_9 (standard check +100:60:80 kg NPK). The *Azotobacter* isolate AZOT-B-33 significantly increased the percent N content in fruit over standard check when the plants were fertilized with the NPK level of 100:60:80. However, the N content due to isolates 33 and standard check were at par with the N content of fruits of uninoculated plants grown with 120:60:80 kg NPK (C-I). The nitrogen content in the fruits of plants raised with the 115:60:80 NPK level was statistically comparable to the

Table 1. Nitrogen fixation capacity of local *Azotobacter* isolates and standard check in the N free Jensen's liquid medium

Azotobacter Isolates	Percent N	N – fixed (mg N /gm of sucrose)
Standard check	0.0262	13.10
AZOT-B-33	0.0269	13.45
AZOT-B-32	0.0263	13.15
AZOT-B-18	0.0261	13.05
AZOT-B-39	0.0218	10.90
AZOT-B-123	0.0209	10.45
AZOT-B-35	0.0207	10.35
AZOT-B-109	0.0203	10.15
AZOT-B-46	0.0200	10.00
AZOT-B-51	0.0198	9.90
AZOT-B-126	0.0197	9.85
AZOT-B-144	0.0196	9.800
AZOT-B-34	0.0195	9.75
AZOT-B-156	0.0195	9.75
AZOT-B-146	0.0087	4.34
AZOT-B-121	0.0053	2.62
Rest isolates	0.0047-0.0052	2.35-2.60
CD (p=0.05)	0.0008	0.40

nitrogen content resulting from the inoculation of local *Azotobacter* isolates 18, 32, 39, and 123 with the C-III fertilizer level. Similar results were found by Reddy et al. (2018). The maximum growth of tomato was in T6, which consisted of 75% of the NPK dose along with *Azotobacter* sp. and *Azospirillum* sp. The observed parameters in T6 were germination 90%, plant height 51 cm, leaf area 59 cm², branches per plant 8.66, and leaves per branch 17.33. The study indicate that these strains possess great potential to be developed as biofertilizers to enhance soil fertility and plant growth.

Fruit nitrogen uptake: The inoculation of tomato plants with *Azotobacter* isolates including standard check significantly increased the accumulation of nitrogen by the fruits except isolate AZOT-B-109. The isolate No 33 showed the best performance which was able to uptake 797.26 mg nitrogen per pot in presence of 100:60:80 NPK level followed by the uninoculated fertilization (716.94 mg/pot) containing 120:60:80 kg of NPK (C-I). Minimum N- uptake by fruits was in uninoculated control treatment with 100:60:80 NPK level (C-III). The *Azotobacter* isolate AZOTO-B-33 significantly increased nitrogen uptake in tomato fruits over standard check of *Azotobacter* with the same level of NPK i.e. 100:60:80. The local isolate (33) alone was at par result with control-I which fertilized with 120:60:80 kg of NPK. The amount of nitrogen which was up taken by fruits due to inoculation with three other local *Azotobacter* isolates AZOT-B-18,32 and 39 and fertilization with 100:60:80 kg NPK was at par with nitrogen accumulated under uninoculated fertilizer treatment C-II (115:60:80) . Inoculation of standard check was found significantly superior over uninoculated control C-II. A similar type of study was also conducted by Reddy et al. (2022) on the production of growth substances by nine *Azotobacter chroococcum* isolates from the sugar beet rhizosphere. This study showed that these isolates have the ability to produce auxins, gibberellins, and phenols, and, in association with tomato plants, increased plant length, biomass, and nitrogen content. *Azotobacter* application significantly increased nitrogen uptake in tomato. This was attributed to better nitrogen fixation resulting from accelerated activity of *Azotobacter* and enhanced root system development, which likely led to higher nitrogen accumulation in tomato shoots (Kumar et al., 2023).

Table 2. Influence of *Azotobacter* isolates and different levels of nitrogen on N-accumulation by tomato fruits and shoot at harvest (percent)

Treatment number	Treatment	Fruits	Shoot
T_1	100:60:80 + AZOT-B-35	1.49	0.64
T_2	100:60:80 + AZOT-B-32	1.77	0.73
T_3	100:60:80 + AZOT-B-18	1.72	0.72
T_4	100:60:80 + AZOT-B-39	1.66	0.70
T_5	100:60:80 + AZOT-B-123	1.60	0.67
T_6	100:60:80 + AZOT-B-33	1.95	0.79
T_7	100:60:80 + AZOT-B-109	1.28	0.60
T_8	N:P:K::100:60:80 + *S.C.*	1.78	0.73
T_9	N:P:K::120:60:80 (C-I)	1.91	0.77
T_{10}	N:P:K::115:60:80 (C-II)	1.69	0.72
T_{11}	N:P:K::100:60:80 (C-III)	1.06	0.52
	CD (p=0.05)	0.16	0.05

Shoot nitrogen content: The local *Azotobacter* isolates, standard check and different levels of nitrogen exhibited a differential influence to enhance shoot N content of tomato plants, (Table 2, 4). The inoculation of tomato seedlings with local *Azotobacter* isolates and standard check with 100:60:80 NPK significantly increased the N-content in tomato shoot at the time of harvest over uninoculated control C-I (100:60:80). Maximum percent N content in shoot was 0.79 % in local *Azotobacter* isolate AZOT-B-33 followed by uninoculated control C-I (0.77%). Minimum value was recorded in C-III (100:60:80) i.e. 0.52%. The *Azotobacter* isolate AZOT-B-33 significantly increased the percent N content in shoot over standard check when the plants were fertilized with the NPK level of 100:60:80. The level of N due to isolate 33 and standard check was at par with the nitrogen content in uninoculated plants raised under NPK level of 120:60:80 (C-I). Nitrogen content in shoot under another uninoculated control treatment C-II (NPK::115:60:80) was statistically insignificant over local *Azotobacter* isolates AZOT-B-18, 32, 39 and 123 with CIII fertilizer level.

Shoot nitrogen uptake: The inoculation of tomato plants with *Azotobacter* isolates including standard check significantly increased the accumulation of nitrogen by the shoot at the time of harvest. Maximum accumulation of nitrogen in plant shoot was attributed to the inoculation of local *Azotobacter* isolate AZOT-B-33 (595.19 mg/pot) with 100:60:80 NPK level, followed by uninoculated fertilized pot (542.57 mg/pot) containing 120:60:80 kg NPK. Minimum N-uptake was under uninoculated control treatment with 100:60:80 NPK level (C-III). The *Azotobacter* isolate AZOT-B-33 significantly increased the nitrogen uptake in tomato shoots over standard check of *Azotobacter* with the same level of NPK i.e. 100:60:80. However, promising isolate (33) and standard check were at par with that of control–I which received only 120:60:80 kg NPK. The amount of nitrogen which was accumulated by *Azotobacter* isolates AZOT-B-18,32,39 & 123 in presence of 100:60:80 kg NPK was statistically equal to that of nitrogen accumulation under

uninoculated fertilizer treatment C-II (115:60:80 NPK). The increment of nitrogen in tomato shoots may be attributed to N-fixation or glutamase synthetase activity. This observation is in close agreement with Mahato et al. (2009) and Tian et al. (2022). They clearly mentioned that *Azotobacter* inoculation either individually or in combination with other crop beneficial microbe significantly increased nitrogen concentration in the root, shoot and whole plant, hence showed better results as compare to that of inorganic fertilizer.

Total nitrogen uptake: The inoculation of tomato seedlings with local *Azotobacte*r isolates and standard check significantly enhanced the total nitrogen uptake by the crop. The maximum amount of N was accumulated by tomato crop (1392.44 mg/pot) due to inoculation of local *Azotobacter* isolate AZOT-B-33 followed by uninoculated treatment C-I (1259.52 mg/pot) with 120:60:80 NPK level. Significant increase in N-uptake by tomato crop varied from 341.10 (C-III) to 1392.44 mg/pot (AZOT -B-33), The local *Azotobacter* isolate AZOT-B-33 alone significantly superior over control treatment C-I and standard check. However, the control treatment C-I was significantly superior over standard check. *Azotobacter* isolates AZOT-B-32 and 18 were par with control treatment C-II (115:60:80 NPK). The study indicate that nitrogen accumulation in the crop was increased by inoculation with local *Azotobacte*r isolates and standard check. The isolate AZOT-B-33 was most effective inoculant for enhancing fruit yield of tomato and the plant nitrogen accumulation. The possible mechanisms that facilitated greater nitrogen uptake by crops include nitrogen fixation (N_2), the delivery of combined nitrogen to the plant, production of phytohormone-like substances that alter plant growth and morphology, and bacterial nitrate reduction, which increases nitrogen accumulation in inoculated plants (Zhang et al., 2022). Verma et al. (2019) also reported that the presence of *Azotobacter* increased nitrogen content in plants rather than phosphorus.

Interaction with *Fusarium*: Out of seven local *Azotobacter* isolates studied, four have shown complete inhibition of the growth of the pathogen (*Fusarium oxysporium*) (Table 2, 3, 4 and Plate 1). The standard check has also shown complete suppression of the fungus. The promising local isolates of *Azotobacter* AZOT-B-33 and 32 were most effective for hundred percent inhibition of *Fusarium oxysporium* (Plate 5). Two other local isolates (AZOT-B-18 and 123) also exhibited hundred percent performance to control *Fusarium oxysporium.* The three local *Azotobacter* isolate AZOT-B-35, 39 and 109 although were significantly superior over control with respect to inhibition of fungal growth but were inferior to isolate AZOT-B-32, 18, 123, 33 and standard check.

Table 3. Effect of different local isolates & standard check of *Azotobacter*on *Fusarium oxysporium*

Azotobacter isolates	*Fusarium oxysporium* growth in millimeters (mm)
AZOT-B-35	18.00
AZOT-B-32	00.00
AZOT-B-18	00.00
AZOT-B-39	12.00
AZOT-B-123	00.00
AZOT-B-33	00.00
AZOT-B-109	15.00
Standard check	00.00
Control	90.00
CD (p=0.05)	1.05

Table 4. Influence of *Azotobacter* isolates and different levels of nitrogen on N-accumulation by tomato fruits and shoot at harvest ((mg / pot)

Treatment	Treatment	Fruits	Shoot	Total (fruit+ shoot)
T_1	100:60:80 + AZOT-B-35	238.43	381.27	619.71
T_2	100:60:80 + AZOT-B-32	512.91	496.68	1009.59
T_3	100:60:80 + AZOT-B-18	430.97	487.14	918.12
T_4	100:60:80 + AZOT-B-39	391.71	433.94	825.65
T_5	100:60:80 + AZOT-B-123	322.45	407.89	730.35
T_6	100:60:80 + AZOT-B-33	797.26	595.19	1392.44
T_7	100:60:80 + AZOT-B-109	174.22	340.84	515.06
T_8	N:P:K::100:60:80 + *S.C.*	610.88	503.33	1114.22
T_9	N:P:K::120:60:80 (C-I)	716.94	542.57	1259.52
T_{10}	N:P:K::115:60:80 (C-II)	466.83	486.69	953.52
T_{11}	N:P:K::100:60:80 (C-III)	105.76	235.33	341.10
CD (p=0.05)		96.93	90.01	102.32

Plate 1. Antifungal activity of promising *Azotobacte*r isolates and standard check in dual culture against *Fusarium oxysporium*

Mahalakshmi and Reetha (2009) also observed that six out of nine isolates of *Azotobacter* of tomato rhizosphere positive towards IAA production, phosphate solubilization, siderophore production, HCN production, ACC deaminase activity and antifungal activity. The two above isolates were effective in inhibiting the growth of fungal pathogen *Fusarium oxysporium*, causing wilt of tomato. Sharma et al. (2023) also reported that seed bacterization with *Rhizobium* inhibited the growth of *Sclerotium* spp. The fungal inhibition by *Rhizobium* isolates may be due to production of secondary metabolites with antimicrobial activities under different environment (Kaur and seema, 2002). Similarly, *Rhizobium* and *Bradyrhizobium* strains were also significantly suppress the mycelial growth of *Fusarium* and other soil-borne pathogenic fungi under in vitro conditions (Verma et al. 2022, Singh et al., 2023, Kumar et al., 2023, Patel et al., 2023). Keeping in view of above mentioned findings, local *Azotobacter* isolate AZOT-B-33 was the most effective isolate for tomato as its inoculation showed best results. The performance of local *Azotobacter* isolate AZOT-B-33 was also significantly superior over standard check to increase yield, dry matter accumulation and nitrogen uptake by tomato crop. However, the performance of both AZOT-B-33 and standard check was at par with CI (120:60:80 NPK level), which means that these organisms were able to supplement 20 kg nitrogen per hectare. *Azotobacter* isolates AZOT-B-32 and 18 were also efficient to save 15 kg of mineral nitrogen per hectare. Similar views were also expressed by Yadav et al. (2023).

CONCLUSION

The local Azotobacter isolate AZOT-B-33 was identified as the most effective for improving nitrogen content and uptake in tomato plants, as well as for inhibiting *Fusarium oxysporum*. It outperformed the standard check and other local isolates in enhancing plant growth and nitrogen utilization. About 20 kg nitrogen could be saved per hectare by using the above bio-inoculant. The findings suggest that AZOT-B-33 could be a valuable biofertilizer for tomato cultivation, potentially reducing the need for chemical fertilizers and improving disease resistance.

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Influence of Land Use Practices on Soil Extractable Iron and Manganese

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Abstract: Land use changes may influence the availability of iron (Fe) and manganese (Mn) in soil. Therefore, the present study was undertaken to assess the influence of land use changes on the extractability of Fe and Mn by different chemical reagents. Four land use practices such as rice-rice, rice-fallow, rice-green gram and uncultivated fallow were chosen for the study. Soil samples were collected from these land use practices and the Fe and Mn contents were extracted with different extractants (DTPA, AB-DTPA, Mehlich-3 and HCl). The land use practices highly influenced the extractability of soil Fe and Mn. Mehlich-3 extracted higher amount of Fe and Mn compared to other extractants across the land use practices. The HCl and Mehlich-3 extractants extracted on average 1.9 and 3.8 and 1.9 and 2.5 times more Fe and Mn than those extracted with DTPA extractant, respectively. Across the land use practices, AB-DTPA and DTPA extractants extracted similar magnitude of Fe and Mn. Among the four land use practices, the rice-green gram system always had the highest amount of extractable Fe and Mn compared to others. Significant positive correlations were observed among the extractants which is a testament to the fact that these extractants could extract Fe and Mn from similar pools. Pearson correlations showed significant positive correlations between organic carbon and extractable Fe and Mn. The inclusion of soil properties in the regression equation resulted improved predictability of extractable soil Fe and Mn.

Keywords: Land use practices, DTPA, AB-DTPA, Mehlich-3, HCl, Extractable Fe and Mn

Iron (Fe) and manganese (Mn) are the essential micronutrients required for the growth and development of plants (Alejandro et al., 2020, Rai et al., 2021, Li et al., 2023). Iron plays important role in nucleic acid metabolism (Ciosek et al., 2023), synthesis and maintenance of chlorophyll in plants (Ning et al., 2023), activates large number of enzymes etc (Ciosek et al., 2023). Manganese participates in the photosynthesis (water splitting enzymes associated with P.S-II) (Alejandro et al., 2020) and detoxification of superoxide free radicals by synthesizing superoxide dismutase (Li et al., 2017) besides playing important role in tricarboxylic acid cycle in oxidative and non-oxidative decarboxylation reaction. Due to these immense roles in plant metabolism, the deficiency of Fe and Mn affects chlorophyll formation, plant growth and grain yield (Moreira et al., 2018). About 19.2 and 17.4% of Indian soils are deficient in Fe and Mn respectively (Shukla et al., 2021). Iron and manganese exist in different chemical pools in soils and their bioavailability for plant nutrition is influenced by the soil properties (Mogta and Sharma, 2018). Soil properties such as pH, redox potential, organic matter content, and mineralogy strongly influence the bioavailability of Fe and Mn for plant nutrition. Further, the bioavailability of these micronutrients may vary with the land use practices. The rice-wheat land use system represents alternate flooding (reduced state) and upland (oxidised state) which may affect the transformation of Fe and Mn in soils influencing their bioavailability. The management practices could alter the distribution of Fe and Mn in soil. Assessing the concentration of micronutrients is highly essential since high levels of Fe and Mn in soils may contribute to secondary contamination of groundwater (Xu and Li, 2024).

Several extractants have been tested for their extraction efficiency and capturing the changes in concentration of Fe and Mn in soils due to management practices. Extractants containing weak acids and weak chelating agents with weak replacement of ions in salts primarily used for predicting plant available forms of micronutrients (Pradhan et al., 2018). Rao et al (2008) reported that extractants used for measuring plant available forms of Fe and Mn vary with mode of action and strength of extraction. Multi-nutrient extractants such as Mehlich-3 and AB-DTPA have also widely been validated in different soil types. However, information on the availability of Fe and Mn under different land use practices is lacking. Hence, the present study was undertaken to assess the extractable Fe and Mn in soils under different land use practices.

MATERIAL AND METHODS

Surface soil samples (0-15cm) were collected from farmers' fields in Bargarh district ($21^0$33'N and $83^0$62'E) of Odisha under four different land uses practices *viz.,* rice-rice, rice-fallow, rice-green gram, and uncultivated fallow. In total, 20 geo-referenced soil samples were collected after the

harvest of rice using a simple random technique. The site falls under the Western Central table land zone of Odisha and is characterized by a hot and moist sub-humid climate. It receives an annual rainfall of ~1,400mm, and more than 90% of the rainfall occurs from June to September. In winter, the minimum temperature goes down to 12°C, while in summer, the maximum temperature goes up to 40°C. Recommended doses of fertilizers were applied for rice while no fertilizer was applied for green gram. The collected soil samples were air-dried, ground, sieved with a 2.0mm sieve, and stored in moisture-proof bags for further analysis of soil properties. Different soil properties and extractable Fe and Mn were analysed following standard protocols (Table 1 and 2).

RESULTS AND DISCUSSION

Soil properties: Soil properties were influenced by the land use practices (Table 3). Soil pH was lowest in rice-green gram (6.48) while the highest in rice-rice (6.78) which was at par with fallow system (6.72). Soil organic carbon content (g kg^{-1}) varied between 6.10 and 7.10 across different land use practices. Compared with the rice-rice system, the fallow, rice-fallow and rice-green gram systems showed an increase in SOC content by 3.3, 6.6, and 16.4%, respectively. Calcium carbonate content (g kg^{-1}) was lowest in rice-green gram system while the highest amount noticed in rice-rice system. Interestingly, there was an increasing trend of $CaCO_3$ content with rising soil pH, while oxides of Fe and Al decreased with increasing pH. The iron oxide content (g kg^{-1}) ranged from 0.807 (rice-rice) to 1.102 (rice-green gram). Similarly, the Al_{ox} content (g kg^{-1}) was found to be in the range of 0.533 to 0.604. The microbial biomass carbon (MBC) which serves as one of the sensitive indicators for change in land use practices varied from 168.4 µg g^{-1} in rice-rice system to 190.3 µg g^{-1} in rice-green gram system accounting 2.76 and 2.68% of SOC respectively. The low pH in the rice-green gram system could be due to the higher content of amorphous Fe_{ox} and Al_{ox}. Higher SOC content in the rice-green gram system may be due to the higher rhizodeposition.

Extractable iron and manganese: Land use practices significantly influenced the extractability of all four extractants (Table 4). The DTPA extractable Fe ranged from 22.4 mg kg^{-1} in the rice-rice system to 28.3 mg kg^{-1} in the rice-green gram system. Irrespective of the extractants tested for extraction of Fe, the lowest amount of extractable Fe was observed in the rice-rice system, while the highest amount was observed in the rice-green gram system. Interestingly,

Table 1. Methods used for analysis of soil properties.

Soil properties	Abbreviation	Unit	References
pH	-	-	Jackson (1972)
Soil organic carbon	SOC	g kg^{-1}	Walkley and Black (1934)
Aluminium oxide	Al_{ox}	g kg^{-1}	McKeague and Day (1966)
Iron oxide	Fe_{ox}	g kg^{-1}	McKeague and Day (1966)
Calcium carbonate	$CaCO_3$	g kg^{-1}	Page et al (1982)
Microbial biomass carbon	MBC	µg g^{-1}	Vance et al (1987)

Table 2. Extractants used for estimation of extractable Fe and Mn in soils under different land use practices

Extractants used	Extractants composition	Soil: extractant ratio	Shaking time	References
DTPA	0.005M DTPA+ 0.01M $CaCl_2$+ 0.1M TEA	1:2	2 hrs	Lindsay and Norvell (1978)
AB-DTPA	1.0M NH_4HCO_3+ 0.5M DTPA (pH 7.6)	1:2	15 min	Soltanpour and Schwab (1977)
Mehlich-3	0.2 MHOAc+0.25M NH_4NO_3 +0.015M NH_4F+0.013M HNO_3+ 0.001M EDTA (pH 2.5±0.1)	1:10	5 min	Mehlich (1984)
HCl	0.1N HCl	1:5	30 min	Osiname et al (1973)

Table 3. Soil properties under different land use practices

Land use practices	pH	SOC	Al_{ox}	Fe_{ox}	$CaCO_3$	MBC
Rice-rice	6.78^{a}	6.10^{d}	0.533^{c}	0.807^{d}	2.08^{a}	168.4^{d}
Rice-fallow	6.63^{b}	6.50^{b}	0.596^{a}	0.918^{b}	1.96^{b}	184.2^{b}
Rice-green gram	6.48^{c}	7.10^{a}	0.604^{a}	1.102^{a}	1.87^{c}	190.3^{a}
Uncultivated fallow	6.72^{ab}	6.30^{c}	0.576^{b}	0.878^{c}	2.03^{a}	173.1^{c}

Different letters (a–d) in each column indicate significant differences between the land use practices according to Duncan's multiple range test ($p<0.05$)
SOC: Soil organic carbon; Al_{ox}: Aluminium oxide; Fe_{ox}: Iron oxide; $CaCO_3$: Calcium carbonate; MBC: Microbial biomass carbon

the HCl and Mehlich-3 extractants obtained on average 1.9 and 3.8 times more Fe than those extracted with DTPA extractant, respectively. Across all land use practices, the order of extractability was: Mehlich-3> HCl> AB-DTPA> DTPA. In case of Mn, the DTPA extractant extracted lowest amount while Mehlich-3 extracted higher amount followed by HCl and AB-DTPA respectively across the land use practices. The highest amount of Mehlich-3 extractable Fe and Mn across land use practices compared to other extractants could be due to the presence of acid reagents and chelating agent such as EDTA. Moreover, the presence of NH_4^+ ion in Mehlich-3 could displace the exchangeable cations (Pradhan et al., 2018). Higher amount of extractable Fe and Mn by Mehlich-3 was also reported from several studies

Table 4. Extractable iron and manganese (mg kg^{-1}) in soils under different land use practices

Land use practices	Iron				Manganese			
	DTPA	AB-DTPA	Mehlich-3	HCl	DTPA	AB-DTPA	Mehlich-3	HCl
Rice-rice	22.4[c]	23.1[c]	84.2[d]	42.2[d]	10.8[d]	11.6[c]	26.4[c]	20.8[d]
Rice-fallow	24.8[b]	25.6[b]	96.1[b]	47.5[b]	11.6[b]	12.4[b]	28.2[b]	22.6[b]
Rice-green gram	28.3[a]	30.2[a]	104.2[a]	53.2[a]	14.2[a]	15.8[a]	36.5[a]	24.6[a]
Uncultivated fallow	24.1[b]	25.4[b]	92.8[c]	45.3[c]	11.2[c]	11.4[c]	27.5[b]	21.6[c]

Different letters (a–d) in each column indicate significant differences between the land use practices according to Duncan's multiple range test ($p<0.05$)

Table 5. Pearson correlation between extractable Fe and soil properties.

	pH	SOC	Al_{ox}	Fe_{ox}	$CaCO_3$	MBC	DTPA_Fe	AB-DTPA_Fe	Mehlich-3_Fe	HCl_Fe
pH	1									
SOC	-0.849**	1								
Al_{ox}	-0.722**	0.774**	1							
Fe_{ox}	-0.825**	0.946**	0.755**	1						
$CaCO_3$	0.829**	-0.935**	-0.841**	-0.882**	1					
MBC	-0.823**	0.895**	0.830**	0.891**	-0.930**	1				
DTPA_Fe	-0.801**	0.951**	0.756**	0.955**	-0.882**	0.903**	1			
AB-DTPA_Fe	-0.825**	0.944**	0.761**	0.970**	-0.893**	0.872**	0.956**	1		
Mehlich-3_Fe	-0.846**	0.913**	0.879**	0.906**	-0.899**	0.923**	0.941**	0.924**	1	
HCl_Fe	-0.821**	0.963**	0.812**	0.946**	-0.924**	0.923**	0.971**	0.955**	0.943**	1

* $p < 0.05$, ** $p < 0.01$
SOC: Soil organic carbon; Al_{ox}: Aluminium oxide; Fe_{ox}: Iron oxide; $CaCO_3$: Calcium carbonate; MBC: Microbial biomass carbon; DTPA_Fe: Diethylene triamine penta acetic acid extractable Fe; AB-DTPA_Fe: Ammonium bicarbonate-diethylene triamine penta acetic acid extractable Fe; Mehlich-3_Fe: Mehlich-3 extractable Fe; HCl_Fe: Hydrochloric acid extractable Fe

Table 6. Pearson correlation between extractable Mn and soil properties

	pH	SOC	Al_{ox}	Fe_{ox}	$CaCO_3$	MBC	DTPA_Mn	AB-DTPA_Mn	Mehlich-3_Mn	HCl_Mn
pH	1									
SOC	-0.849**	1								
Al_{ox}	-0.722**	0.774**	1							
Fe_{ox}	-0.825**	0.946**	0.755**	1						
$CaCO_3$	0.829**	-0.935**	-0.841**	-0.882**	1					
MBC	-0.823**	0.895**	0.830**	0.891**	-0.930**	1				
DTPA_Mn	-0.807**	0.955**	0.672**	0.969**	-0.877**	0.860**	1			
AB-DTPA_Mn	-0.801**	0.922**	0.615*	0.924**	-0.831**	0.845**	0.976**	1		
Mehlich-3_Mn	-0.819**	0.947**	0.635**	0.955**	-0.846**	0.821**	0.990**	0.969**	1	
HCl_Mn	-0.853**	0.948**	0.780**	0.958**	-0.913**	0.926**	0.947**	0.914**	0.928**	1

* $p < 0.05$, ** $p < 0.01$
SOC: Soil organic carbon; Al_{ox}: Aluminium oxide; Fe_{ox}: Iron oxide; $CaCO_3$: Calcium carbonate; MBC: Microbial biomass carbon; DTPA_Mn: Diethylene triamine penta acetic acid extractable Mn; AB-DTPA_Mn: Ammonium bicarbonate-diethylene triamine penta acetic acid extractable Mn; Mehlich-3_Mn: Mehlich-3 extractable Mn; HCl_Mn: Hydrochloric acid extractable Mn

Table 7. Multiple linear regression equation showing the relationship between extractable Fe and Mn and soil properties

Extractable Fe	Regression equation	R^2
DTPA	114.12-(13.4)pH**	0.642
	-13.7+ (0.39)pH+ (5.53)SOC**	0.905
	-16.3+ (0.61)pH+ (5.35)SOC**+ (4.14)Al_{ox}	0.906
	-12.0+(1.19)pH +(2.27)SOC+(2.29)Al_{ox} +(10.4) Fe_{ox}	0.935
	-18.3+ (1.10)pH+ (3.10)SOC+ (3.92)Al_{ox}+ (10.3)Fe_{ox}+ (1.98)$CaCO_3$	0.935
	-44.9 + (1.58) pH + (3.52) SOC + (0.89) Al_{ox} + (7.77) Fe_{ox} + (7.18) $CaCO_3$ + (0.08) MBC	0.946
AB-DTPA	135.6-(16.4)pH**	0.680
	-1.35-(0.16)pH+ (5.93)SOC**	0.892
	-5.13-(1.36)pH+ (5.67)SOC **+ (5.95)Al_{ox}	0.894
	1.84-(0.42)pH+ (1.47)SOC+ (2.93)Al_{ox}+ (17.0) Fe_{ox}*	0.947
	11.4-(0.29)pH+ (0.97)SOC+ (0.44)Al_{ox}+ (17.3) Fe_{ox}* -(3.02)$CaCO_3$	0.948
	26.4-(0.56)pH+ (0.73)SOC+ (2.15)Al_{ox}+ (18.7)Fe_{ox}-(5.96)$CaCO_3$- (0.45)MBC	0.950
Mehlich-3	409.3- (47.3)pH **	0.716
	102.7-(14.2)pH+ (13.2)SOC *	0.851
	38.3- (0.89)pH+ (8.81)SOC+ (101.2)Al_{ox}*	0.914
	46.3-(7.92)pH+ (4.04)SOC+ (97.8)Al_{ox}*+ (19.3)Fe_{ox}	0.923
	33.9- (8.09)pH+ (4.69)SOC+ (101.0)Al_{ox}*+ (19.0)Fe_{ox}+ (3.92)$CaCO_3$	0.923
	-55.6-(6.47)pH+ (6.09)SOC+ (90.8)Al_{ox}+ (10.5)Fe_{ox}+ (21.4)$CaCO_3$+ (0.27)MBC	0.934
HCl	219.0 – (25.8) pH *	0.675
	-16.5 – (0.40) pH + (10.1) SOC**	0.927
	-31.9 + (0.84) pH + (9.13) SOC** + (24.1)Al_{ox}	0.938
	-27.1 + (1.47) pH + (6.29) SOC* + (22.1)Al_{ox} + (11.5) Fe_{ox}	0.948
	-13.4+ (1.66) pH + (5.58) SOC + (18.5)Al_{ox} + (11.8) Fe_{ox} – (4.33) $CaCO_3$	0.949
	-50.3 + (2.33) pH + (6.15) SOC + (14.3)Al_{ox} + (8.38) Fe_{ox} + (2.89) $CaCO_3$ + (0.11) MBC	0.955
Extractable Mn		
DTPA	66.8-(8.24)pH**	0.651
	-10.9+ (0.14)pH+ (3.36)SOC**	0.912
	-5.97- (0.25)pH+ (3.70)SOC**- (7.77)Al_{ox}	0.923
	-2.64+ (0.19)pH+ (1.70)SOC*-(9.21)Al_{ox}*+ (8.15)Fe_{ox}**	0.969
	3.45+ (0.27)pH+ (1.38)SOC- (10.7)Al_{ox}+ (8.31) Fe_{ox}**- (1.92)$CaCO_3$	0.971
	4.31+ (0.26)pH+ (1.37)SOC- (10.6)Al_{ox}+ (8.40) Fe_{ox}**- (2.09)$CaCO_3$-(0.003)MBC	0.971
AB-DTPA	85.2- (10.8)pH**	0.642
	-7.36- (0.88)pH+ (4.01)SOC**	0.852
	2.91- (1.72)pH+ (4.72)SOC**- (16.1)Al_{ox}	0.879
	6.47- (1.24)pH+ (2.58)SOC- (17.7)Al_{ox}+ (8.68) Fe_{ox}	0.909
	7.71- (1.23)pH+ (2.52)SOC- (18.0)Al_{ox}+ (8.71)Fe_{ox}-(0.39)$CaCO_3$	0.909
	-11.7- (0.87)pH+ (2.82)SOC- (20.2)Al_{ox}+ (6.86)Fe_{ox}+ (3.42)$CaCO_3$+ (0.05)MBC	0.917
Mehlich-3	197.1- (25.1)pH**	0.670
	-21.0-(1.61)pH+(9.44)SOC**	0.897
	1.86- (3.47)pH+ (11.0)SOC**- (36.0)Al_{ox}	0.923
	11.0- (2.24)pH+ (5.51)SOC*- (39.9)Al_{ox}*+ (22.1)Fe_{ox}*+ (2.78)$CaCO_3$	0.962
	2.20- (2.36)pH+ (5.97)SOC- (37.6)Al_{ox}*+ (22.1)Fe_{ox}*+ (2.78)$CaCO_3$	0.962
	24.2- (2.76)pH+ (5.63)SOC- (35.1)Al_{ox}+ (24.2)Fe_{ox}* - (1.52)$CaCO_3$-(0.06)MBC	0.964
HCl	85.4- (9.47)pH**	0.727
	15.4- (1.91)pH+ (3.03)SOC	0.906
	12.5- (1.67)pH+ (2.83)SOC+ (4.56)Al_{ox}	0.909
	15.4- (1.27)pH+ (1.04)SOC+ (3.27)Al_{ox}+ (7.27)Fe_{ox}	0.941
	25.8- (1.13)pH+ (0.50)SOC+ (0.59)Al_{ox}+ (7.54)Fe_{ox}*- (3.27)$CaCO_3$	0.944
	11.2- (0.86)pH+ (0.73)SOC- (1.07)Al_{ox}+ (6.15)Fe_{ox}-(0.41)$CaCO_3$+ (0.04)MBC	0.951

* $p < 0.05$, ** $p < 0.01$; SOC: Soil organic carbon; Al_{ox}: Aluminium oxide; Fe_{ox}: Iron oxide; $CaCO_3$: Calcium carbonate; MBC: Microbial biomass carbon

(Rodrigues et al., 2001, Pradhan et al., 2018). Dilute acid (0.1N HCl) may only capable of solubilizing soil Fe and Mn partially (Pradhan et al., 2018). Among the four land use practices, the rice-green gram system always had the highest amount of extractable Fe and Mn compared to others. Moreover, the influence of the rice-green gram system over the rice-rice system was more pronounced when Fe was extracted with AB-DTPA (30.7%), followed by DTPA (26.3%), HCl (26.1%) and Mehlich-3 (23.8%) (whereas in Mn the extractability followed the order of Mehlich-3 (38.3%) followed by AB-DTPA (36.2%), DTPA (31.5%) and HCl (18.3%) respectively (Fig. 1 and 2). High organic C content in the rice-green gram system (Table 3) might be the reason for increase in the availability of Fe and Mn in soils which was captured by the extractants used in the present study. Increase in organic C content in soils could increase the availability of Fe and Mn in soils (Annepu et al., 2017, Siva Prasad et al., 2023).

Pearson correlation matrix was constructed between the extractable Fe and Mn and soil properties to establish their relationships. The extractable Fe and Mn showed significant positive correlations with SOC and MBC while significant negative correlations with soil pH and $CaCO_3$ content (Table 5, 6). DTPA extractable Fe showed significant positive correlation with AB-DTPA (r= 0.956**), Mehlich-3 (r= 0.941**) and HCl (r= 0.971**). Similar correlations were obtained among the extractable Mn. Such dynamic relationships among the extractable Fe and Mn suggested that the extractants could extract Fe and Mn from similar pools. The extractability of different extractants is influenced by soil properties (Table 8). In the case of DTPA extractable Fe, soil pH alone caused a variation of 60.2%, which was improved to 94.6% with the inclusion of other soil properties such as SOC, Al_{ox}, Fe_{ox}, $CaCO_3$ and MBC. Similarly, the variation in Mehlich-3 extractable Fe caused by soil pH alone was 71.6%, and it was improved to 85.1 and 91.4% with the inclusion of SOC and Al_{ox} respectively. Both soil pH and SOC could explain 91.2% of the variability in DTPA extractable Mn. In general, with the inclusion of different soil properties in the regression equation, the prediction of extractable Fe and Mn improved.

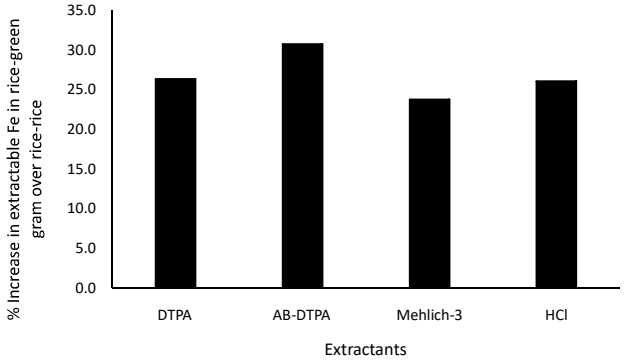

Fig 1. Relative increase in extractable Fe in rice-green gram over rice-rice land use system

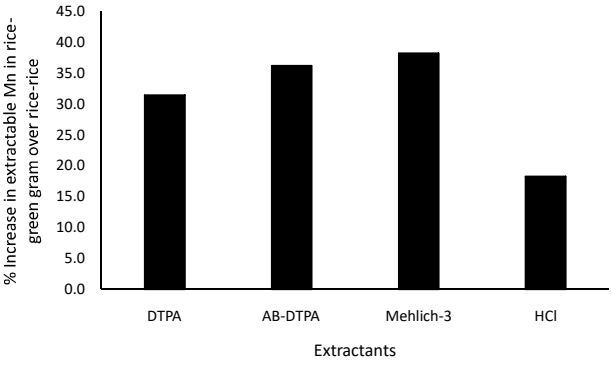

Fig 2. Relative increase in extractable Mn in rice-green gram over rice-rice land use system

CONCLUSION

Land use practices significantly influenced the extractability of Fe and Mn by different chemical extractants. Mehlich-3 extracted higher amount of Fe and Mn while DTPA extracted the lowest amount. Across the land use practices, the order of extractability for Fe and Mn was Mehlich-3> HCl> AB-DTPA> DTPA. Similarly, among the land use practices rice-rice system had lowest amount of extractable Fe and Mn while rice-green gram system showed highest amount of extractable Fe and Mn.

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Seasonal Variations and Land-Use Impacts on Soil Fertility in Riparian Zone of the Dhansiri River, North East, India

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***Abstract*:** Riparian zones are critical for ecosystem functions such as nutrient cycling, erosion control, and flood regulation. However, these zones are increasingly impacted by human activities. This study investigates seasonal variations in soil physicochemical properties across three land-use types in the Dhansiri River Basin, Northeast India. Key soil parameters, including pH, soil temperature, soil moisture, bulk density, soil organic carbon, and nutrients (available nitrogen, available phosphorus, exchangeable potassium), were analysed across four seasons. Statistical analyses revealed significant seasonal and spatial variations in soil properties. Seasonal fluctuations were observed in pH, soil moisture, soil temperature, and nutrient levels, with urban areas showing higher nutrient concentrations due to organic waste deposition. Bulk density and soil organic carbon content varied significantly across land-use types, reflecting the influence of anthropogenic activities. Correlation analysis demonstrated strong positive associations between pH, soil moisture, and nutrient availability, highlighting the impact of seasonal changes and land-use practices on soil fertility. The findings underscore the need for sustainable land management to mitigate anthropogenic impacts and preserve riparian soil health.

Keywords: Riparian zone, Dhansiri River, Soil properties, Seasonal variation, Land use-impacts

The riparian zone denotes the broad area encircling a water body, stretching from the riverbank to the floodplains. The primary definitions of riparian zones emphasize a functional perspective, highlighting the reversible hydrological, morphological, chemical, and biological interactions present in both water and land systems (Majumdar and Avishek 2023). Besides these essential functions, riparian zones provide various ecosystem services and products that enhance human well-being, including livestock feed, genetic resources, flora and fauna for decorative purposes, fuelwood, carbon sequestration, regulation of the air quality, flood mitigation, pollination, recreation, and aesthetic value. In addition, the riparian vegetation prevents riverbanks against erosion, diminishes evaporation, and prevents soil sediments and minerals from runoff (Dufour et al., 2019). However, despite providing several ecological functions and service are most endangered ecosystems globally because of human activities and natural disturbances. Most commonly, agricultural practices, urban development, alteration in river flow, overexploitation, climate change, pollution, and biological invasions are significant hazards that compromise and degrade riparian zones (Singh et al., 2021). The riparian zones of Indian rivers exhibit diverse influences due to considerable changes in temperature, topography, soil, land use, and anthropogenic activities. Survey in 1995 by the Forest Survey of India indicated that 85% of the Ganga River basin is devoid of forest cover Roy et al. (2015). This is chiefly attributable to agriculture, pollution, dam construction, water extraction, logging, tourism, and flooding (Majumdar and Avishek 2023). Given the importance of the riparian zone, a thorough examination of the soil's physicochemical properties conducted across three distinct land use activities within the Dhansiri river basin to assess soil fertility and the impact of seasonal fluctuations on the soil.

MATERIAL AND METHODS

Experimental area: The present study was conducted in the riparian zone of the Dhansiri River Northeast India. Soil samples were taken from December 2021 to November 2022, encompassing the four seasons: winter, spring, summer, and autumn (Fig. 1). Soil samples were collected from three distinct sites within the Dhansiri River basin, each representing different land-use practices, for subsequent analysis. The sites were selected to reflect a range of land-use types in the region.

Rural Site (R-S1): Located in an agricultural area with typical farming practices, latitude 25°45'20"N and longitude 93°34′38″E with elevation 193.53 msl.

Urban Site (U-S2): Situated in an urbanised zone, affected by anthropogenic activities, including waste disposal, sand mining, and infrastructure development, latitude25°55′24″N and 93°44′58″E, with elevation 163.12 msl.

Semi-Rural Site (SR-S3): Impacted by sand mining, and brick kiln operations, latitude 26°13′07″N and 93°50′54″E with elevation 141.54 msl.

Soil analysis: Soil samples were collected from three different locations, placed in airtight bags, air-dried, and sieved to ensure a consistent particle size. Methodology used for analysis soil parameters listed in Table 1.

Statistical analysis: This was performed using SPSS version 21, including correlation analysis to explore the relationships between soil properties .Significant differences were identified at the 5% level using Duncan's Multiple Range Test (DMRT, $p<0.05$).

RESULTS AND DISCUSSION

The mean values of soil physico-chemical parameters from three different study sites are presented in Table 2. The *p*-values and *F*-values for the seasonal variation of soil parameters, are shown in Table 3.

pH: Soil pH peaked at SR-S3 with a mean of 7.15, whereas the lowest was at R-S1 (6.21). The lower pH at R-S1 may be due to the application of compost and manure fertilizers in the topsoil, which can acidify the soil (Temjen et al., 2022). Similar observation was recorded by Yadav et al. (2024), where at higher elevations, low pH was observed, likely due to the leaching of exchangeable bases through runoff and erosion. In all sampling sites and seasons, the soil pH ranged from slightly acidic to neutral. The highest pH was during the summer, while the lowest occurred in winter. Statistically significant differences were observed in all three stations.

Soil temperature (ST): Soil temperature is strongly influenced by seasonal changes, with summer temperatures reaching 33.92°C and winter temperatures dropping to 22.04°C. These fluctuations are further impacted by spatial and temporal variations, as well as the surrounding vegetation density. In particular, the SR-S3 exhibited higher temperatures, likely due to its lower elevation and the depletion of vegetation caused by sand mining activities. Statistical analysis revealed the ST differences at this site were significant,.

Soil moisture (SM): At all sampling points, SM was consistently lower due to the predominance of sandy soil, which exhibits high permeability and thus reduced moisture retention capacity compared to other soil types. SM content was highest during the rainy season i.e. summer (17.46%), which concurrently diminished in the winter (7.97%). The

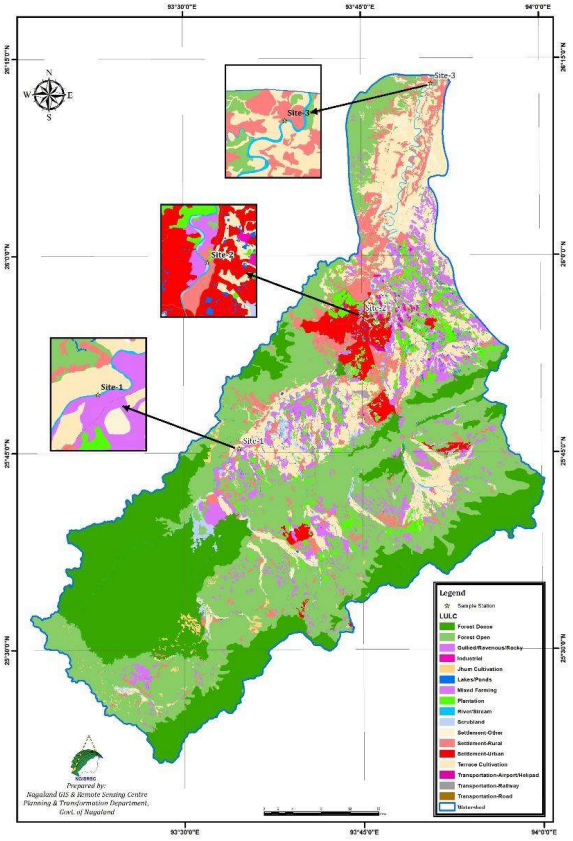

Fig. 1. Land use and land cover map of the three-sampling point of the Dhansiri river

Table 1. Methodology and instruments used to analysis the soil parameters

Parameters	Method	Reference/Instrument
pH	Glass electrode method	Glass electrode
Soil temperature (ºC)	Soil thermometer	Soil thermometer
Soil moisture (%)	Gravimetric method (weighing before and after drying)	Oven, Analytical balance
Bulk density (g cm^{-3})	Core sampler method	Core sampler, Oven, Analytical balance
Conductivity (µS cm^{-1})	Electrical conductivity meter (1:5 w/v, distilled water).	Electrical conductivity meter
Organic carbon (%)	Walkley and Black method (titration method)	Walkley and Black, 1934
Exchangeable potassium (kg ha^{-1})	Flame photometry (photometric method)	Flame photometer
Available nitrogen (kg ha^{-1})	Kjeldahl method (distillation and titration method)	Kelplus Nitrogen Estimation system
Available phosphorus (kg ha^{-1})	Bray's no. 1 extraction method	Bray's no.1, 1945; Spectrophotometry
Soil texture (%)	Pipette method for particle size distribution	Piper, 1942

Table 2. Seasonal mean values of the physicochemical properties of soil of the three sites with Duncan's multiple range test (p<0.05)

	Seasons	pH	ST (°C)	SM (%)	BD (g cm^{-3})	EC (µS cm^{-1})	SOC (%)	K_{ex} (Kg ha^{-1})	N_{av} (Kg ha^{-1})	P_{av} (Kg ha^{-1})	Sand (%)	Silt (%)	Clay (%)
R-S1	Winter	$5.7±0.01^{a}$	$22.68±0.50^{a}$	$8.75±0.61^{a}$	$1.48±0.03^{b}$	$206.44±6.86^{a}$	$0.2±0.01^{a}$	$187±2.92^{a}$	$67.37±5.38^{a}$	$7.43±0.28^{a}$	$82.8±0.33^{b}$	$10.26±0.53^{a}$	$5.97±0.22^{a}$
	Spring	$6.2±0.07^{b}$	$26.5±1.22^{b}$	$11.74±0.34^{b}$	$1.43±0.04^{b}$	$228.47±2.32^{b}$	$0.22±0.01^{a}$	$212.04±5.60^{b}$	$86.27±3.44^{b}$	$8.34±0.42^{ab}$	$81.19±0.66^{a}$	$11.83±0.62^{bc}$	$6.63±0.33^{ab}$
	Summer	$6.5±0.01^{c}$	$32.6±1.16^{d}$	$16.85±0.49^{c}$	$1.34±0.03^{a}$	$226.97±0.71^{b}$	$0.3±0.02^{b}$	$238.55±2.72^{c}$	$106.84±4.52^{c}$	$10.13±0.28^{c}$	$82.7±0.7^{a}$	$11.24±0.48^{c}$	$6.61±0.25^{ab}$
	Autumn	$6.2±0.13^{b}$	$29.38±1.43^{c}$	$12.68±1.89^{b}$	$1.43±0.01^{b}$	$218.95±4.05^{b}$	$0.32±0.03^{b}$	$220.27±5.73^{b}$	$92.04±3.30^{bc}$	$8.93±0.36^{bc}$	$81.21±0.22^{ab}$	$11.76±0.11^{ab}$	$6.92±0.13^{b}$
Mean±S.E.		6.15±0.10	27.79±0.68	12.5±0.57	1.42±0.01	220.21±1.86	0.26±0.01	214.47±3.45	88.13±3.21	8.71±0.21	81.97±0.33	11.27±0.28	6.53±0.12
U-S2	Winter	$6.1±0.09^{a}$	$22.27±0.33^{a}$	$7.97±0.68^{a}$	$1.42±0.01^{c}$	$212.89±3.25^{a}$	$0.2±0.02^{a}$	$174.46±2.50^{a}$	$81.78±3.98^{a}$	$9.39±0.34^{a}$	$82.8±0.33^{a}$	$10.26±0.53^{a}$	$6.94±0.28^{ab}$
	Spring	$6.51±0.1^{b}$	$26.59±1.23^{b}$	$12.18±0.66^{b}$	$1.38±0.02^{bc}$	$246.42±2.01^{b}$	$0.22±0.02^{a}$	$185.71±7.56^{a}$	$101.6±8.10^{b}$	$10.55±0.02^{ab}$	$81.22±0.66^{a}$	$11.81±0.64^{b}$	$6.97±0.54^{b}$
	Summer	$7.11±0.09^{c}$	$33.68±0.62^{d}$	$17.4±1.1^{c}$	$1.32±0.02^{a}$	$249.06±5.34^{b}$	$0.39±0.04^{b}$	$216.98±5.11^{b}$	$141.45±5.80^{c}$	$12.73±0.23^{c}$	$82.72±0.72^{a}$	$11.22±0.51^{ab}$	$6.05±0.31^{a}$
	Autumn	$6.57±0.24^{b}$	$30.3±2.43^{c}$	$13.01±2.25^{b}$	$1.37±0.02^{b}$	$215.56±1.41^{a}$	$0.25±0.03^{a}$	$204.86±7.17^{b}$	$110.46±7.99^{b}$	$11.57±0.62^{bc}$	$81.25±0.18^{a}$	$11.71±0.07^{ab}$	$7.03±0.12^{b}$
Mean±S.E.		6.57±0.13	28.21±0.80	12.64±0.67	1.37±0.01	230.98±3.28	0.26±0.01	195.5±3.37	108.82±4.10	11.06±0.26	81.99±0.29	11.25±0.22	6.75±0.14
SR-S3	Winter	$6.05±0.14^{a}$	$22.04±0.82^{a}$	$8.25±0.81^{a}$	$1.39±0.02^{b}$	$186.54±3.50^{a}$	$0.21±0.01^{a}$	$185.5±9.70^{a}$	$84.55±1.41^{a}$	$9.24±0.31^{a}$	$82.2±0.12^{a}$	$11.57±0.13^{a}$	$6.23±0.18^{ab}$
	Spring	$6.54±0.09^{b}$	$27.92±1.59^{b}$	$12.8±0.50^{b}$	$1.36±0.03^{b}$	$207.57±3.85^{b}$	$0.27±0.03^{b}$	$207.36±15.79^{b}$	$107.59±8.12^{b}$	$11.16±0.07^{b}$	$80.94±0.36^{a}$	$12.3±0.18^{a}$	$6.75±0.21^{ab}$
	Summer	$7.15±0.13^{c}$	$33.92±0.23^{c}$	$17.27±0.63^{c}$	$1.26±0.01^{a}$	$202.98±0.63^{b}$	$0.32±0.01^{bc}$	$253.77±6.78^{c}$	$116.11±5.24^{b}$	$12.62±0.51^{c}$	$82.59±0.68^{a}$	$11.57±0.53^{a}$	$5.83±0.16^{a}$
	Autumn	$6.79±0.2^{b}$	$29.87±1.99^{b}$	$13.22±2.2^{b}$	$1.23±0.03^{a}$	$200.57±3.42^{ab}$	$0.35±0.01^{c}$	$221.39±8.81^{b}$	$106.67±5.33^{b}$	$12.19±0.28^{bc}$	$80.62±0.63^{a}$	$12.09±0.37^{a}$	$7.28±0.54^{b}$
Mean±S.E.		6.62±0.14	28.44±0.81	12.88±0.66	1.31±0.01	199.41±2.24	0.29±0.01	217±4.95	103.73±2.74	11.30±0.30	81.59±0.29	11.88±0.17	6.52±0.16

The mean values in column with superscript (a,b,c,d) are significantly different at 5% level by Duncan's multiple range test (p<0.05)

difference was seen at all three sites.

Bulk density (BD): The average means recorded in R-S1, U-S2, and SR-S3 were 1.42g cm^{-3}, 1.37g cm^{-3}, and 1.31g cm^{-3}, respectively. The maximum BD of 1.48g cm^{-3} was in winter at R-S1. Similar findings was reported by Takele et al. (2014). Minimum value of 1.26 g cm^{-3} was observed in summer at SR-S3. In R-S1 and U-S2 and SR-S3 statistical difference was observes at p<0.001.

Electrical conductivity (EC): The average EC in R-S1, U-S2, and SR-S3 varied from 199.41 to 230.98 µScm^{-1}. The minimum concentration of EC was in winter at SR-S3 (1876.54 µScm^{-1}) and maximum in summer at U-S2 (249.06 µScm^{-1}), which attributed to the increased concentration of salts from various chemicals, dissolved solids, trace metals, colloidal particles, and ions, hence enhancing soil conductivity (Tewari et al., 2016). The three sites demonstrate significant seasonal variation.

Soil organic carbon (SOC): Soil Organic Carbon plays a crucial role in maintaining soil health, contributing significantly to soil fertility, productivity, and overall ecosystem functionality (Ebabu et al., 2020). The SOC content was highest in U-S2 (0.39 %). Similar observation of high OC content in dumping site was observed by Agbeshie et al., (2020) and lowest in R-S1 (0.19 %), with the oxidation of organic carbon being exacerbated by practices such as shifting agriculture, overgrazing, and leaching near the riparian zones. These factors contribute to a decrease in SOC. Additionally, seasonal fluctuations in SOC were significant at p<0.001 indicating variation over time in all three sites.

Exchangeable potassium (K_{ex}): Potassium is crucial for various physiological processes in plants and is an essential element for their development. The mean K_{ex} in R-S1, U-S2, SR-S3 soil was 214.47, 195.5 and 217±4.95 kg ha^{-1}. The maximum was in S3 (253.77 kg ha^{-1}), while the minimum in U-S2 (174.46kg ha^{-1}). The diminished quantity of potassium may result from extensive garbage disposal, causing deterioration and loss of vegetation in that region. The comparable pattern was corroborated by Njue et al. (2016). Seasonally, the maximum K_{ex} was in summer and minimum in winter across all three land types. K_{ex} exhibited variation across all three sites.

Available nitrogen (N_{av}): Available nitrogen is an essential element of soil quality, significantly influencing plant growth, agricultural yield, and overall ecosystem functionality (Prasad et al., 2023). There were significant seasonal variations in the N_{av} across three distinct soil samples. During summer, the soil exhibited the maximum N_{av} level in U-S2 (141.45kg ha^{-1}), followed by SR-S3 and R-S1. Similar findings were observed by Ramya et al. (2021). The

elevated N_{av} levels can be attributed to the deposition of nutrient-rich organic matter in the dumping zones, which enhances the population of nitrogen-fixing organisms, as well as to higher biological nitrogen fixation. This is in agreement with Saha et al. (2018).

Available phosphorous (P_{av}): Seasonally fluctuation was observed with the maximum P_{av} in summer (12.62-12.73 kg ha^{-1} in SR-S3 and U-S2). The concentration of P_{av} was minimum in winter (7.43kg ha^{-1}). Significant difference was observed among the seasons. Among the three sites, the P_{av} concentration was higher in U-S2 and SR-S3. The high moisture content in U-S2 and SR-S3, along with the presence of organic carbon and microbial activities in the dumping area, could be the reason for the increased availability of phosphorus and other nutrients in these sites compared to R-S1. Semy and Singh (2021) reported similar findings.

Soil texture: The textural analysis of the three study areas revealed that the soils were primarily loamy sand. The sand content ranged from 82.62 to 82.80%, silt from 10.26 to 12.30%, and clay from 5.83 to 7.28%, with variations observed across the four seasons. The significant seasonal variation in sand and silt content was in R-S1 with the highest sand percentage in winter and the lowest in spring and autumn. Silt content peaked in spring SR-S3 and autumn for SR-S3, while silt was lowest in winter at all sites. No significant seasonal variations in sand, silt, and clay concentration were observed in U-S2 and SR-S3. Meanwhile, in all three sites highest clay content occurred in autumn, with the lowest levels recorded in summer. These

Table 3. Seasonal variation of the three-study area

Parameters	R-S1		U-S2		SR-S3	
	p-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value
pH	< 0.001	25.81	< 0.001	26.45	< 0.001	32
ST	< 0.001	46.09	< 0.001	41.37	< 0.001	42.48
SM	< 0.001	28.73	< 0.001	21.70	< 0.001	22.99
BD	< 0.001	10.54	< 0.001	16.40	< 0.001	26.82
EC	< 0.001	13.63	< 0.001	32.12	0.003	5.71
SOC	< 0.001	24	< 0.001	23.00	< 0.001	19.18
K_{ex}	< 0.001	50.07	< 0.001	23.19	< 0.001	26.72
N_{av}	< 0.001	13.30	< 0.001	38.83	< 0.001	11.39
P_{av}	0.001	11.61	< 0.001	12.45	< 0.001	17.49
Sand	< 0.001	9.26	0.043	3.05	0.031	3.34
Silt	<0.001	10.21	0.038	3.15	0.319	1.22
Clay	0.58	2.77	0.034	3.27	0.12	4.25

Table 4. Correlation among the different soil parameters

	PH	ST	SM	BD	EC	SOC	K_{ex}	N_{av}	P_{av}	SAND	SILT	CLAY
pH	1											
ST	.951**	1										
SM	.981**	.954**	1									
BD	-.911**	-.871**	-.854**	1								
EC	.694*	.615*	.690*	-.468	1							
SOC	.786**	.841**	.741**	-.804**	.462	1						
K_{ex}	.911**	.969**	.896**	-.873**	.539	.849**	1					
N_{av}	.959**	.978**	.950**	-.850**	.669*	.794**	.971**	1				
P_{av}	.929**	.962**	.941**	-.828**	.650*	.845**	.895**	.932**	1			
Sand	.061	-.034	.022	-.035	-.104	.156	.065	.037	-.097	1		
Silt	.284	.366	.315	-.337	.371	.120	.314	.323	.372	-.851**	1	
Clay	-.201	-.072	-.192	.187	-.150	-.145	-.140	-.143	-.019	-.857**	.544	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

findings suggest that the soils have low porosity and poor water retention capacity, with sand being the dominant particle size at all sites. This observation is consistent with the results of Singh et al., (2016) in soils of Punjab. The reduced presence of clay and silt particles may be linked to the effects of grazing animals, which can cause the removal of finer particles through wind and water erosion (Hishe 2017).

Correlation: The Pearson correlation analysis indicate a very strong positive association between pH and SM, with a correlation coefficient of r=0.981, which is statistically significant (Table 4). This signifies that as pH rises, SM also ascends in a nearly linear manner. In addition, pH showed a positive corelation with ST, SOC, N_{av}, P_{av}, and K_{ex}, obtaining significance at $p<0.01$, indicating that fluctuations in pH values will appropriately influence the rise or a decline of specific parameters. Semy and Singh (2021) also observed a substantial negative correlation between BD and pH. ST exhibited a positive significance with SM, SOC, and NPK. Jiao et al. (2016) reported a similar observation, increase in ST elevates nutrient levels in the soil. The ST was found to have a negative correlation with BD. SM was strongly and significantly positively correlated with micronutrient concentration NPK and with EC and SOC at $p<0.05$. BD exhibits a negative connection with, NPK at $p<0.01$. The majority of the parameters did not exhibit a significant relationship with EC, with the exception of N_{av} and P_{av}, which was positively significant. Substantial positive correlation was observed between SOC and NPK. The SOC exhibited a significant positive correlation with NPK, presumably as available nutrients are integral components of organic matter. The quantity of organic matter and its mineralization by microorganisms play a critical role in the availability of nutrients in soils (Singh et al., 2012). SOC was negatively correlated with BD, which can be attributed to the high organic matter present. Similar findings were reported by Ruiz Sinoga et al. (2012). The micronutrients NPK exhibited positive correlations with each other, as well as with pH, ST, and SM. NPK showed a negative correlation with BD. Sand content was negatively correlated with both silt and clay

CONCLUSION

This study highlights the negative impact of agricultural expansion, landfills, and sand mining on the Dhansiri riparian zone. Clearing vegetation and poor land management have degraded soil quality, reducing fertility and ecosystem services. Soil properties show seasonal variation, with lower bulk density in summer and moderate to low organic carbon levels. Despite some increases in nutrients (NPK), the overall soil health has declined, reflecting the effects of population growth and development. The findings emphasize the need for sustainable land use and riparian conservation to restore soil fertility and ecosystem function.

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Study on Combining Ability and Gene Action in Pigeonpea (*Cajanas cajan* (L.) Millsp.) For Grain Yield and Quality Traits in Humid South Eastern Plain Climate Zone

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Abstract: Twenty-eight hybrids developed by utilizing eight parents in 8 x 8 diallel mating design excluding reciprocals were evaluated in randomized block design (Agriculture University, Kota during, 2023-24) for thirteen characters in order to understand the combining ability and gene action in pigeonpea. The analysis of variance for combining ability revealed presence of non- additive gene action. The ratio of gca/sca variance was less than unity which indicated the preponderance of non- additive gene action for action. The estimates of general combining ability suggested that parents ICPL-20338, ICPL-20340 and Pusa-992 were good general combiners for seed yield per plant and attributing characters while, hybrids ICPL-20338 x ICPL-20340, ICPL-20338 x AL-882, ICPL-20340 x AL-882 and Pusa-992 x PA-16 showed the higher order sca effect for seed yield per plant. These cross combinations can be potentially utilized in hybrid breeding programmes.

Keywords: Combining ability, Non-additive gene effect, Pigeonpea, Diallel mating design

Pigeonpea [*Cajanus cajan* (L) Millsp.] is an often-cross-pollinated important grain legume crop mainly grown under rain-fed conditions in India. Therefore, pigeonpea can be improved genetically following breeding methods suitable for both allogamous as well as autogenous crops. Pigeonpea differs from other legumes as it exhibits large variation (20-70%) in natural out crossing, it can be considered as an often-cross-pollinated crop. Selection of parent genotypes together with information on nature and magnitude of gene action controlling grain yield and its attributing characters is prerequisite while improving the plant type. The study on combining ability provides useful information on selection of parents in terms of performance of their hybrids and elucidates the nature and magnitude of various types of gene action involved in the expression of the quantitative traits. The limited studies on the combining ability contribute to limited selection of the best genotypes for specific traits in pigeonpea. The objective of this combining ability analysis in early genotypes of pigeonpea.

MATERIAL AND METHODS

Eight diverse cultivars of pigeonpea, namely, ICPL-20338, ICPL-20340, ICPL-87, Pusa-991**,** Pusa-992, PA-16, PA-291 and AL-882 were crossed in all possible combinations excluding reciprocals in *Kharif* 2022-23. The resulting F_1's, and eight parents were grown in a randomized block design replicated thrice at the farm attached to the agriculture research station, Agriculture university, Kota during *kharif* 2023-24. Five randomly selected competitive plants from each genotype were used in recording observations on the characters including days to 50% flowering, days to maturity, plant height, primary branches per plant, pod per plant, seed per pod, pod length, 100 seed weight, biological yield per plant, grain yield per plant, harvest index, protein content and carbohydrate content. Combining ability estimates of parents and crosses were estimated according to the Method-2, Model-1 of Griffing (1956).

RESULTS AND DISCUSSION

The significant and high variances observed for all the thirteen characters revealed the genetic variability among the genotypes. The variance due to SCA was higher than the variance due to GCA for all the traits including seed yield per plant showed the predominance of dominance gene action for these traits. these characters could be improved by heterosis breeding. However, σ^2gca/ σ^2sca ratio being less than unity indicating that the non-additive gene action was more important in the expression of all characters in each environment (Table 1) Similar results were reported in earlier studies(Shekhar et al., 2004, Banu et al., 2006, Kumar et al., 2009, Satpute et al., 2019).

The gca effects of parents revealed that the parents ICPL-20338, ICPL-20340 and Pusa-992 were good general combiners for seed yield and its direct components (Table 2). ICPL-20338 was good general combiner for primary branches per plant, pods per plant, seeds per pod, and biological yield per plant. ICPL- 20340 for pods per plant, seeds per pod, 100-seed weight and biological yield per plant and Pusa-992 for pods per plant, 100-seed weight, biological

yield per plant and protein content. Pusa-991 and PA-16 were good combiners for earliness and dwarfness.

The estimation of specific combining ability effects revealed that cross combinations ICPL-20338 x ICPL-20340, ICPL-20338 x AL-882, ICPL-20340 x AL-882 and Pusa-992 x PA-16 exhibited significant and positive sca effects for seed yield per plant resulted from good x good, good x poor, good x poor and good x average parents, respectively. Better performance of hybrids involving high x low or low x low general combiners indicated dominance x dominance type of gene interaction. The crosses showing high sca effects involving one good general combiner indicated additive x dominance type gene interaction which exhibited the high heterotic performance for yield and yield related traits. These

Table 1. Analysis of variance for combining ability for yield and its contributing traits

Characters	Source of variance					
	GCA (7)	SCA (28)	Error (70)	σ^2gca	σ^2sca	σ^2gca/ σ^2sca
Days to 50% flowering	4.76**	10.84**	0.72	0.40	10.12	0.03
Days to maturity	3.72**	6.89**	0.95	0.28	5.94	0.04
Plant height	3.72**	6.89**	0.95	3.34	54.88	0.04
Primary branches per plant	0.76**	2.95**	0.19	0.06	2.75	0.02
No. of pods per plant	1454.19**	1236.34**	38.52	141.57	1197.82	0.11
No. of seed per pod	0.22**	0.22**	0.04	0.02	0.18	0.09
Pod length	0.24**	0.24**	0.05	0.02	0.19	0.10
100- seed weight	0.63**	0.78**	0.10	0.05	0.68	0.07
Biological yield per plant	177.25**	405.88**	17.63	115.96	388.25	0.29
Seed yield per plant	52.73**	86.64**	3.86	4.89	82.78	0.05
Harvest index	16.65**	33.38**	3.28	1.34	30.10	0.04
Protein content	1.43**	4.41**	0.20	0.12	4.21	0.02
Carbohydrate content	22.27**	32.09**	0.49	2.18	31.61	0.06

*,** Significant at 5 and 1 per cent level

Table 2. Estimates GCA effects for different traits in pigeonpea

Parents	DFF	DM	PH	PBPP	NPPP	NSPP	PL	100-SW	BYPP	GYPP	HI	PC	CC
ICPL-20338	-0.28	0.48	0.91	0.34*	17.58**	0.18**	0.12	-0.06	11.0**	2.89**	-0.21	-0.69**	-0.02
ICPL-20340	-0.18	0.71*	3.14*	0.14	9.05**	0.19**	-0.16*	0.22*	6.06**	1.30*	-0.33	0.12	-0.52*
ICPL-87	-0.01	-1.15**	-2.31	0.35*	-18.7**	-0.03	0.13	-0.06	-9.35**	0.66	2.83**	-0.13	1.33**
Pusa-991	-0.61*	-1.05**	-3.34	-0.11	-4.12*	-0.1**	0.21**	-0.39**	-16.86**	-4.37**	0.16	0.10	0.60**
Pusa-992	0.25	0.21	-1.71	0.12	8.30**	0.09	0.06	0.23*	12.39**	1.40*	-1.22*	0.51**	-0.76**
PA-16	-0.75**	-1.14**	3.11	0.02	2.25	0.17*	-0.03	-0.31**	1.97	0.69	-0.10	0.28*	-2.49**
PA-291	1.48**	0.37	-2.19	-0.33*	-14.6**	-0.14*	-0.20**	0.24*	-10.42**	-2.15**	0.24	-0.33*	-0.58**
AL-882	0.11	-0.48	2.38	-0.44**	6.33**	-0.16*	-0.13*	0.13	5.14**	-0.43	-1.37*	0.13	2.45**
SE	0.251	0.288	1.838	0.131	1.836	0.065	0.066	0.097	1.242	0.581	0.535	0.132	0.207
gi-gj	0.380	0.436	2.779	0.199	2.775	0.098	0.100	0.146	1.877	0.879	0.809	0.200	0.312

*,** Significant at 5 and 1 per cent level

DFF	-Days to 50% flowering	100-SW	-100 Seed weight
DM	-Days to maturity	BYPP	-Biological yield per plant
PH	-Plant height	SYPP	-Seed yield per plant
PBPP	-Primary branches per plant	HI	-Harvest index
NPPP	-Number of pods per plant	PC	-Protein content
NSPP	-Number of seeds per pod	CC	-Carbohydrate content
PL	-Pod length		

Table 3. Estimates SCA effects for different traits in pigeonpea

Crosses	DFF	DM	PH	PBPP	NPPP	NSPP	PL	100-SW	BYPP	GYPP	HI	PC	CC
ICPL-20338 × ICPL-20340	5.81**	1.63	11.60*	1.16**	34.83**	0.43*	0.73**	1.44**	16.4**	10.49**	4.55 **	0.61	-0.70
ICPL-20338 × ICPL-87	-5.34**	-0.33	0.16	-0.01	14.64*	0.18	0.16	-0.14	11.85**	6.12**	1.65	1.07*	5.79**
ICPL-20338 × Pusa-991	1.25	1.86*	6.29	2.22**	-20.9**	-0.13	-0.21	-0.15	4.29	-5.67**	-5.13**	-1.33**	6.17**
ICPL-20338 × Pusa-992	-1.28	-0.86	1.09	-0.03	15.79**	-0.37	-0.51*	-0.65*	-6.40	5.53**	5.19 **	-2.19**	-7.41**
ICPL-20338 × PA-16	-4.48**	0.83	-11.96*	-0.45	-55.5**	-0.33	-1.19**	1.40**	-14.27**	-12.85**	-6.47 **	-1.29**	0.40
ICPL-20338 × PA-291	2.05*	1.49	-12.71*	-3.70**	40.31**	0.70**	0.30	0.17	22.52**	8.61**	2.30	1.51**	2.40**
ICPL-20338 × AL-882	2.15**	0.97	8.71	1.19**	29.45**	0.93**	0.45*	1.46**	15.40**	11.14**	5.25**	2.54**	3.45**
ICPL-20340 × ICPL-87	-0.78	-6.22**	5.35	-3.71**	-41.1**	-0.35	-1.09**	1.08**	-0.63	-11.70**	-9.19 **	-0.19	3.64**
ICPL-20340 × Pusa-991	-2.18**	0.97	-11.34	0.93*	40.05**	0.31	0.20	-0.43	11.92**	12.81**	6.89 **	0.07	8.16**
ICPL-20340 × Pusa-992	0.95	0.23	-14.04*	0.67	33.65**	0.50*	-0.04	-0.90	8.63*	6.93**	3.60*	3.94**	-5.00**
ICPL-20340 × PA-16	2.75**	3.60**	-12.07*	-3.33**	-67.3**	-0.08	0.52*	-1.02**	-46.89**	-10.25**	0.83	0.46	-2.44**
ICPL-20340 × PA-291	0.28	0.27	6.08	-0.18	1.39	-0.32	-0.38	-0.91**	-13.42**	-6.61**	-2.90	-1.12**	-9.44**
ICPL-20340 × AL-882	3.05**	1.35	11.70**	0.46	45.51**	0.83**	0.92**	1.67**	20.69**	13.33**	5.77**	-1.10*	8.01**
ICPL-87 × Pusa-991	1.31	0.99	-7.48	1.08*	19.33**	-0.06	0.19	-0.08	5.32	-6.05**	-7.44 **	3.43**	2.66**
ICPL-87 × Pusa-992	2.45**	0.93	3.93	0.26	-17.58**	-0.14	-0.19	0.27	12.54**	-7.34**	-7.44 **	0.05	0.05
ICPL-87 × PA-16	0.91	1.96*	9.85	0.57	68.90**	0.61**	0.20	0.06	37.72**	11.61**	0.78	0.45	1.34*
ICPL-87 × PA-291	2.78**	2.63**	5.34	-0.01	-24.90**	-0.03	0.20	-0.86	-20.03**	0.98	6.15**	-0.22	6.15**
ICPL-87 × AL-882	-4.11**	-5.56**	-1.40	0.42	-34.68**	-0.7**	0.24	-0.22	-21.29**	-9.38**	-4.43*	-1.21**	-0.71
Pusa-991 × Pusa-992	2.71**	1.13	-4.27	-1.17**	0.68	0.08	0.14	0.14	13.19**	-0.93	-3.12	4.37**	-3.36**
Pusa-991 × PA-16	-0.81	0.83	5.54	0.08	-8.87	-0.20	-0.15	0.36	-14.16**	-5.23**	-2.22	1.81**	0.57
Pusa-991 × PA-291	-0.61	0.83	-4.93	0.14	-24.39**	0.03	-0.19	0.37	-14.6**	-2.02	0.52	-2.93**	-1.79**
Pusa-991 × AL-882	0.48	1.63	-9.39	-3.75	2.81*	0.08	-0.11	-0.29	20.40**	-5.91**	-7.56 **	-1.01*	-0.61
Pusa-992 × PA-16	-0.01	0.76	6.93	-1.64**	41.05**	0.50*	0.45*	0.87**	14.51**	8.85**	3.77*	-0.82	4.57**
Pusa-992 × PA-291	-3.48**	-2.23*	17.27**	-0.14	19.43**	0.32	0.33	0.90**	14.90**	7.75**	3.12	-0.99*	-7.29**
Pusa-992 × AL-882	-4.38**	-0.76	6.01	1.84**	3.38	0.15	0.14	0.60	-1.72	4.76*	3.94*	-2.17**	6.06**
PA-16 × PA-291	2.98**	-0.20	-12.64*	1.17**	-4.21	0.09	0.48*	0.55	-9.31*	-0.16	1.82	2.25**	5.52**
PA-16 × AL-882	3.75**	-0.06	-6.43	0.21	-11.35	-0.54*	-0.47*	-1.69**	1.84	-8.34**	-5.61 **	-2.25**	-5.25**
PA-291 × AL-882	4.95**	3.60**	2.94	-0.22	13.33*	-0.24	-0.32	0.44	16.84**	1.08	-1.77	-1.20**	3.34**
S.E	0.77	0.88	5.63	0.40	5.62	0.19	0.20	0.29	3.80	1.78	1.64	0.40	0.55
gi-gj	1.14	1.30	8.33	0.59	8.32	0.29	0.30	0.44	5.63	2.63	2.42	0.60	0.63

*,** Significant at 5 and 1 per cent level

combinations also had the higher order sca effects for the number of pods per plant, number of seeds per pod, pod length, 100- seed weight, biological yield per plant and harvest index.

The hybrids Pusa-991 x Pusa-992 (4.37), ICPL-20340 x Pusa-992 (3.94) and ICPL-87 x Pusa-991 (3.43) exhibiting significant and positive sca effects for protein content resulted from average x good, average x good and poor x average parents, respectively. Hybrids ICPL-20340 x Pusa-991(8.16), ICPL-20340 x AL-882 (8.01) and ICPL-20338 x Pusa-991 (6.17) exhibited significant and positive sca effects for carbohydrate t resulted from poor x average, average x good and poor x average parents, respectively. The estimation of specific combining ability effects revealed that cross combinations ICPL-87 x AL-882 and Pusa-992 x PA-291 exhibited significant and negative sca effects for days to 50% flowering and days to maturity (Table 3). These finding were also in confirmation with earlier studies (Mhasal et al., 2015, Yamanura et al., 2016, Moses et al., 2020, Patel et al., 2020, Chandra et al., 2024).

CONCLUSION

The variance due to SCA was higher than the variance due to GCA for all the traits including seed yield per plant showed the predominance of dominance gene action for these traits. However, σ^2gca/ σ^2sca ratio being less than unity indicating that the non-additive gene action was more important in the expression of all characters in each environment. The gca effects of parents revealed that the parents ICPL-20338, ICPL-20340 and Pusa-992 were good general combiners for seed yield and its related traits. Involving these parents in multiple crossing programmes may be developed for isolating high yielding lines. The estimation of specific combining ability effects revealed that cross combinations ICPL-20338 x ICPL-20340, ICPL-20338 x AL-882, ICPL-20340 x AL-882 and Pusa-992 x PA-16 exhibited significant and positive sca effects for seed yield per plant and yield contributing traits. These crosses may be further studies for commercial exploitation of hybrid vigour.

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Calibration and Validation of Integrated Sensors System with Low-Cost Data Acquisition System for Measuring Soil Compaction and Electrical Conductivity of Soil in Central Plane Zone of Punjab

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Abstract: In the present study, an experimental analysis was carried out to find the calibration curves for the S-type load cell used for measuring soil compaction and electrical conductivity measuring sensor based on the Wenner Array method and integrate the sensor's calibrated output corresponding to different depth intervals by using ultrasonic depth sensor after its calibration. The data acquisition system was designed with the help of an Arduino Uno Microcontroller based on ATmega 328P which was equipped with high-performance AVR technology. The monochrome graphic flat panel display Module with 4 Pin was used for displaying the sensor's output data in real-time. A data logger was used for recording and saving the pre-calibrated data from all the integrated sensors in Excel format for accurate calibration of all the integrated sensors. The developed dual soil sensor was validated in the field in three types of tillage treatments in sandy loam soil. The accuracy of the developed dual soil sensor measuring soil compaction and soil electrical conductivity was 95.4 and 73.42 %, respectively.

Keywords: S-type load cell, Electrical conductivity, Ultrasonic depth sensor, Arduino Uno, Calibration, Soil sensors

In India, the majority of people rely on agriculture for their living. Soil conditions have a significant impact on cost-effective and efficient farm production. In order to evaluate the fertility status or the physical and chemical characteristics that affect a soil's suitability for growing plants, soil testing is a crucial technique to carry out. Over time, various soil assessment techniques have been used. In precision agriculture, the lack of soil data that is pertinent, trustworthy, economical, readily accessible, and adequately precise continues to be a major concern. In order to practice precision agriculture, it is necessary to have access to timely, affordable, and accurate soil data (Kumar and Masrat Mohi 2024, Kumar 2024a). Usually, soil sampling and subsequent laboratory analysis, which is very time-consuming and labor-intensive, yield the most crucial soil properties. If performed on a fine grid, soil sampling, and laboratory analysis take a long time and become prohibitively expensive. Testing the soil directly in the field, such as by using real-time soil sensors, may be an alternative to or a supplement to laboratory soil analyses (Adamchuk 2011).

Due to their robust nature, which makes them reliable and suitable for field applications, soil sensors continue to be widely used as the foundation of precision agriculture for mapping soil variability. Before releasing any method for use in practice, these types of sensors need careful calibration and a well-designed validation. Calibration refers to the evaluation of testing equipment (sensors) against the pre-known values by developing the correlation between the actual values and measured values to reduce error in the sensors' measurement. The difference between the expected and measured values of a sensor is called structural error. During the calibration process the set of adjustments performed on a sensor or instrument to make that instrument function as accurately, or error-free, as possible(Soni et al 2017).

In India, soil compaction (MPa or kPa) and electrical conductivity (mSm^{-1}) are measured by penetrometer and laboratory methods, respectively. Soil compaction is commonly used as a measure of soil strength as determined by a cone penetrometer in terms of cone index. There are three different types of electrical conductivity measuring sensors based on the principle of the soil sensor: electromagnetic induction (EMI), time-domain reflectometry (TDR), and direct contact method (ERM). One of the basic elements of electrical conductivity measurement is the electrode array, which characterizes the configuration settings of the electrode sensor used to measure current or voltage. Furthermost, soil sensors based on the electrical resistivity principle such as the Wenner array method are affordable and easy to use (Kumar 2023, Kumar 2024b). Many researchers are working on the development of new improved penetrometers with the incorporation of electronic instruments to monitor the penetration resistance simultaneously with electrical conductivity by employing load

cell, electrical conductivity measuring sensors, and penetration depth using ultrasonic depth sensors. Gradually data loggers and control panels are also introduced in new editions of penetrometers by different researchers (Kumar and Bector 2022).

The S-type load cell is a transducer that can measure force and weight by converting tensile and compressive forces in the form of electrical signals. When the force is applied to the load cell, the shape of the wire is slightly distorted and returns to its original shape, unless it is overloaded, resulting in a change in resistance. This change in resistance is proportional to the pressure of the applied force. The maximum pressure that it can withstand is called the capacity of the load cell **().**The ultrasonic depth sensor triggers an ultrasonic wave from the transmitter at a frequency of 40Hz and the receiver module receives the returned wave signals. Once the wave returns after being reflected by any object, at the same time the Echo pin makes a transition from low to high level for a specified period, which is equivalent to the time it takes for the wave to return to the sensor. The distance is calculated from the speed and time travelled by the wave (Carrara 2014).These sensors are generally interfaced with a microcontroller that monitors such as Arduino Uno, performs transformation, and makes decisions on the sensor's input and output signals. It functions as a small computer, accepting inputs and controlling outputs for various electronic devices (Anonymous 2015).The accuracy of calibration and validation of the sensors in well-designed ecosystems is a key factor in determining the validity of these sensors that are interfaced with the microcontroller. Consequently, objective was to precisely calibrate and validate the integrated sensor system in the actual field using establishedstandard laboratory techniques in real-world settings.

MATERIAL AND METHODS

The major sensors selected for the development of the sensor-based integrated system for measuring soil compaction and electrical conductivity of the soil were an S-type load cell, an ultrasonic depth sensor, and electrical conductivity measuring sensor based on the Wenner Array method.The detailed procedure for the calibration of the integrated sensors systems is presented under the following sub-heading:

Load cell calibration: The S-type load cell of 500 kg capacity was calibrated by applying known dead weight (kg) load vertically under a static load experiment set on the top of the load cell in two phases and the output was recorded in millivolt (Fig. 1). Initially the dead weight ranged from 5 to 50 kg at an interval of 5kg and in the second phase the dead weight ranged from 1 to 50 kg at an interval of 1 kg (Fig. 2, 3). The same procedure for calibration was followed by Rahai (2013), Anonymous (2014), and Anonymous (2017). The signals generated from the load cell were very low and were not able to work with the microprocessor therefore, a signal amplifier cum signal conditioning module (HX711 amplifier module) was used. The signal amplifier provided well-controlled regulated excited voltage to the circuit of the load cell and in addition, it includes noise signal filtering, amplification, and output signal manipulation.

The S-type load cell was connected to the recording unit via a voltage amplifier module (HX711 amplifier module) powered with the 12-volt DC battery. The output measured data without load on the load cell was set at zero and the corresponding output data was recorded with gradually loading and unloading the dead weight on the top of the load cell. The analog to digital (A/D) converted output of the load cell was recorded manually as displayed on the OLED screen. The output of the amplified signals from the load cell

Fig. 1. Experimental setup for load cell calibration with loading and unloading known dead weights

was programmed by using the linear regression equation (1) to express the load from millivolt to kilogram and the basic program of the converted output of the penetration resistance or soil compaction (Cone index), was expressed in megapascal by using the standard equation (2) which states that the cone index is the force (N) that is applied on the base area of the cone. The relationship between the actual load in kilogram and measured output average mean of loading and unloading in millivolt was found to be highly linear at their respective interval and the linear regression line was fitted to the measured output data with R^2 value of 0.99 (Table 1).

Weight (kg) = 2.24028 × (X) + constant

PR = Penetration resistance

X = Analoag to digital converted output (mV)

Constant = 0.628

$$\text{Cone index (MPa)} = \frac{\text{Weight (kg)} \times 9.8}{\text{Cone base area (mn^2)}}$$

Ultrasonic depth sensor calibration: The ultrasonic depth sensor selected was an electronic device that was sensitive to changes under different environmental conditions and thus produce undesirable outputs under abrupt changes. To reduce the error in the measured output and expected output, the sensor was calibrated. The two pins (tripper and echo) were connected to the I/O pins of the microcontroller (Arduino Uno) and readings were displayed on the OLED screen integrated with the circuitry of the ultrasonic sensor and microprocessor (Arduino Uno). The output reading of the ultrasonic sensor displayed on the screen was manually recorded against the actual reading on the measuring tape by placing a target plate (obstacle) in parallel and gradually moving the targeted plate from 0 to 60cm at an interval of 2 cm. The inbuilt circuitry of the ultrasonic sensor automatically calculated the distance by measuring the time taken by the ultrasonic wave when triggered by the trigger pin at 40 Hz and receiving the return wave reflected by the obstacle plate attached parallel to the ultrasonic sensor. The average data from the output values against the actual value at each respective interval indicated almost excellent linearity (Fig. 4, Table 2). The experimental results of the depth sensor showed negligible error as compared to the actual distance. Since the error was very small, therefore it was easily

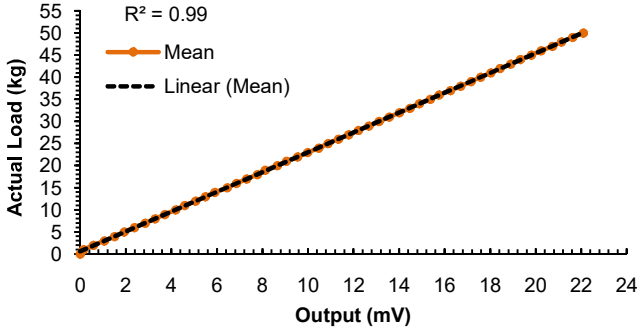

Fig. 3. Calibration of the S-type load cell with loading and unloading known dead weights (1kg interval)

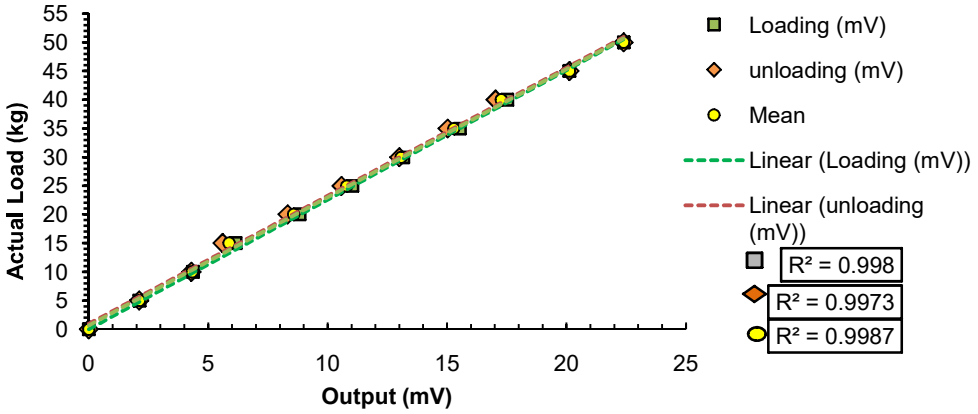

Fig. 2. Calibration of the S-type load cell with loading and unloading known dead weights (5kg interval)

Table 1. Model summary of regression correlation between input and output of actual and measured values of the load cell

Statistical parameters	Load cell range (5-50 kg) at 5 intervals (Actual input value vs output mean)	Load cell range (1-50 kg) at 1 interval (Actual input value vs output mean)
R^2 value (correlation)	0.999	0.999
R^2 value (coefficient of determination)	0.9987	0.999
Adjusted R^2	0.9986	0.999
Standard error of the estimate RMSE	0.3912	0.1086
Linear equation, Y	y = 2.2322x + 0.6836	Y = 2.2402x+0.628

corrected in the programming code.

Electrical conductivity measuring sensor calibration: The electrical conductivity of the soil is directly proportional to the concentration of salt in the soil. High concentrations of ions increase conductivity in which current is transported via ions within the soil. Similarly, the lower the salt concentration, the higher the resistance, and vice versa. For calibrating the developed soil EC sensor, a total of 45 samples of the soil were collected from three different types of locations with different soil textures up to 60 cm at an interval of 20 cm and the samples were air dried (Table 3). The electrical conductivity of each sample was measured as per the standard laboratory process which served as a reference point for calibrating the developed integrated soil sensor system.

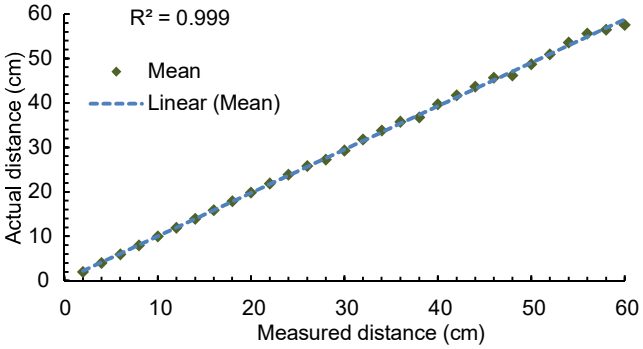

Fig. 4. Calibration graph of the ultrasonic depth sensor

Similarly, the soil electrical resistance was measured from the same location at each point of sample collection for the same soil at an interval of 20 cm up to 60 cm (Fig. 5). The relationship was developed between the electrical conductivity (dSm^{-1}) measured by the standard laboratory methods and the developed sensor's voltage (mV) (Fig. 6 a,b,c). The relationship was also developed from the corresponding output of all the samples with the laboratory methods to establish the overall correlation with all the three types of soil (Fig. 6 d, Table 4). The regression showed an inverse relation between the laboratory method and output from the developed sensing system which was analysis-backed Ohm's law. The same procedures were followed by many researchers (Park et al 2016, Jusoh and Osman 2017). The developed sensor with the microprocessor (Arduino Uno) set up was carried out for the determination of soil

Table 2. Model summary of regression correlation between input and output of actual and measured values of the ultrasonic depth sensor

Statistical parameters	Actual measurement vs output measurement
R^2 value (correlation)	0.9996
R^2 value (coefficient of determination)	0.9992
Adjusted R^2	0.9992
Standard error of the estimate RMSE	0.4662
Linear equation, Y	Y = 0.9764x + 0.255

Fig. 5. Measurement of output data of the developed probe sensor

Table 3. Physical characteristics of selected soil and location of plots

Particulars	Soil type-I	Soil type-II	Soil type-II
Texture class	Sandy silt loam	Sandy loam soil	Sandy Clay loam
Composition of soil texture (Sand, Silt & Clay %)	37.98, 45.61, 16.41	61.07, 27.13, 11.80	70, 13.8, 21.2
Location	L1: Research farm of DFMPE, Near PRSC, Gate no.6, PAU, Ludhiana	L2: Research farm of DFMPE, Near Gate no.4, PAU, Ludhiana	L3: Soil research farm of DSS, Near Gate no.8, PAU, Ludhiana
GPS Coordinates	75° 48'45.06" E, 30° 54'41.04" N	75° 49'08.19" E, 30° 54'38.78" N	75° 52'.10" E, 30° 56'.04" N

electrical conductivity in terms of voltage. This developed system was powered by a portable 12 V battery to make it convenient to handle independently. The sensor produced an output voltage according to the change in the resistance offered by the soil within the soil profile which was recorded manually from the display on the OLED screen. The best-fit regression curve from the regression analysis was drawn which was used for calibrating the soil sensor.

Field evaluation of the integrated soil sensor system : The developed soil sensor system (DSS) was evaluated in three types of tillage treatments (T1, T2, T3). The total percentage of accuracy between the parameters, measured by the developed soil sensor, and the reference parameters measured by the manual hand-held cone penetrometer for cone index (MPa), and laboratory analysis for electrical conductivity of soil (dS/m), was determined by making suitable assumptions that the reference/controlled methods of measuring CI and EC are 100 % accurate. However, the comparison of the data values of CI and EC obtained from the soil sensor developed can be done with values obtained from any other selected method.

RESULTS AND DISCUSSION

Validation of the integrated soil sensor system with a manual cone penetrometer: The accuracy of the sensor was 95.4 %. Similar trends in the data obtained using a manual cone penetrometer as a control method and the integrated soil sensor system's measurements of soil compaction were observed. The highest and lowest mean cone index that the designed integrated soil sensor system measured were 4.0604 and 0.8212 MPa, respectively whereas the highest and lowest mean cone index measured with the hand-held cone penetrometer was 0.9184 and 3.6627 MPa, respectively (Fig. 7). There was a non-significant difference between the soil compaction measured by the developed soil sensor DSS and the manual cone penetrometer (CI MCP) in sandy loam soil (Fig. 8). The R^2 value (correlation) was 0.83 and the coefficient of variation was 12.20, whereas the standard error of the estimate RMSE was 0.322. A box and whisker plot was plotted for displaying the distribution of soil compaction patterns in all three treatments with the developed soil sensor (CI DSS) and the manual hand-held con penetrometer (CI MCP) (Fig. 9).

Validation of the integrated soil sensor system with laboratory methods (EC): The developed soil sensor (EC DSS) was evaluated in three types of tillage treatments (T1, T2, T3) and compared with the laboratory method (EC Lab/control method) in sandy loam soil. Similar trends between data (EC) obtained using a laboratory approach (control method) and data obtained using the developed

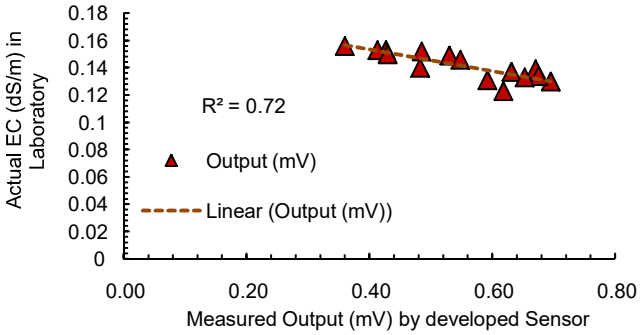

Fig. 6a. Calibration graph for EC measurement at Research Farm of FMPE, PAU, Ludhiana (L1)

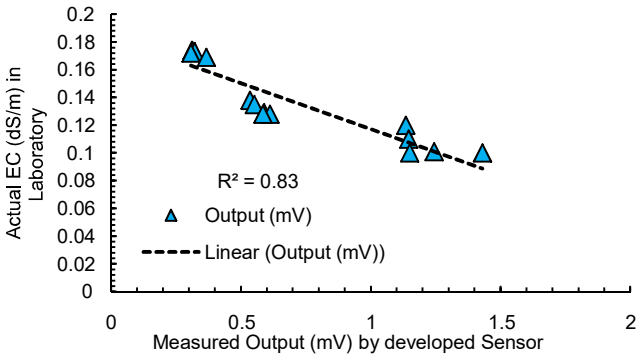

Fig. 6b. Calibration graph for EC measurement at Research Farm of FMPE, PAU, Ludhiana,(L2)

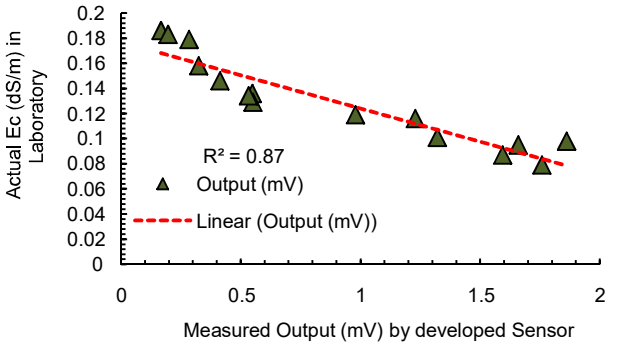

Fig. 6c. Calibration graph EC measurement at Research Farm of Dept. of soil science, Ludhiana (L3)

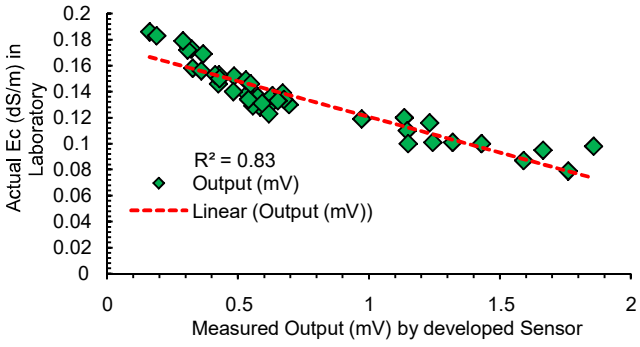

Fig. 6d. Calibration graph of EC measurement output of all three textures of soil at PAU, Ludhiana (L1:L2:L3)

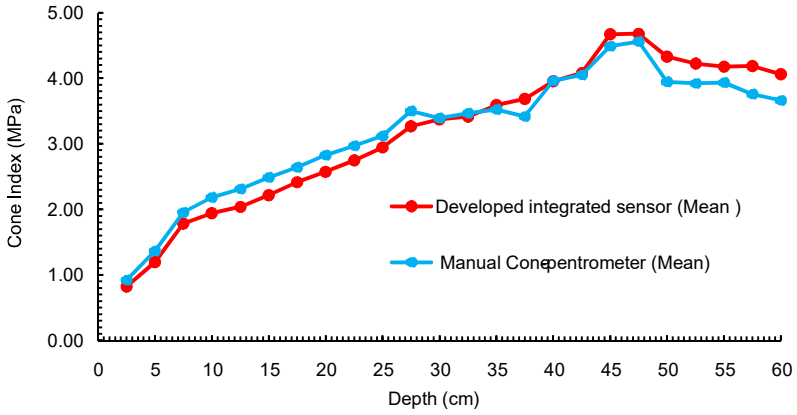

Fig. 7. Measurment of soil compaction

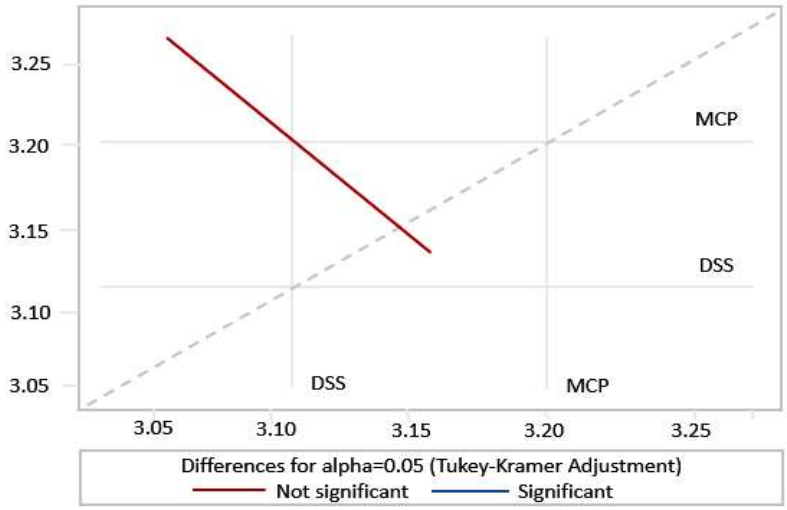

Fig. 8. Diffogram for the effect of methods of measuring soil compaction (DSS and MCP) in sandy loam soil

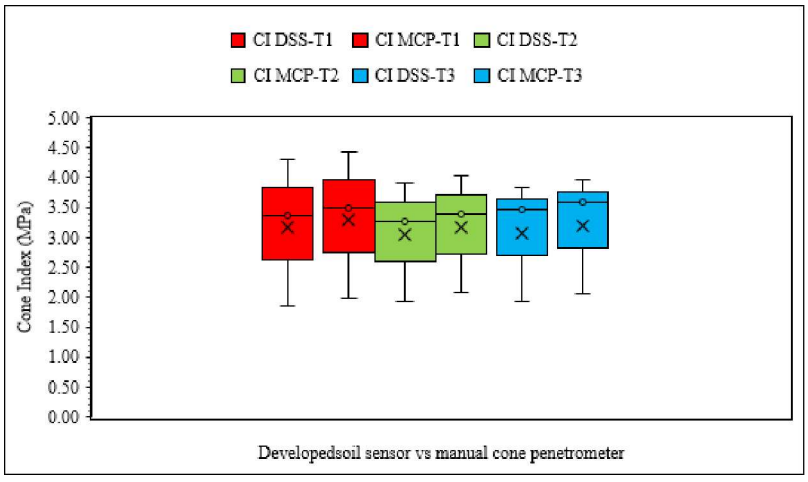

Fig. 9. Whisker's boxplot chart for soil compaction distribution by developed soil sensor (DSS) and manual control penetrometer (MCP) in T1, T2, and T3

integrated soil sensor. The mean of electrical conductivity measured using the developed integrated soil sensor system was 0.0904, 0.0862, and 0.0784 dS/m from 0-20 cm, 20-40 cm, and 40-60 cm, respectively. The electrical conductivity measured using the laboratory method was 0.1276, 0.1150, and 0.1045 dS/m from 0-20 cm, 20-40 cm, and 40-60 cm, respectively (Fig. 10). The accuracy of the sensor was 73.42 %. However, most of the literature revealed the accuracy of real-time EC sensors between 55 to 70 % (Liu et al 2017, Salam et al 2017). There was a significant difference between the soil compaction measured by the developed soil sensor (EC DSS) and the laboratory/control method (EC Lab) in sandy loam soil (Fig. 11). The R^2 value (correlation) was 0.93 and the coefficient of variation was 7.380, whereas the standard error of the estimate RMSE was 0.0074. A box and whisker plot was plotted for displaying the distribution of soil electrical conductivity pattern in all three treatments with the developed soil sensor (EC DSS) and the laboratory method (EC Lab) (Fig. 12).

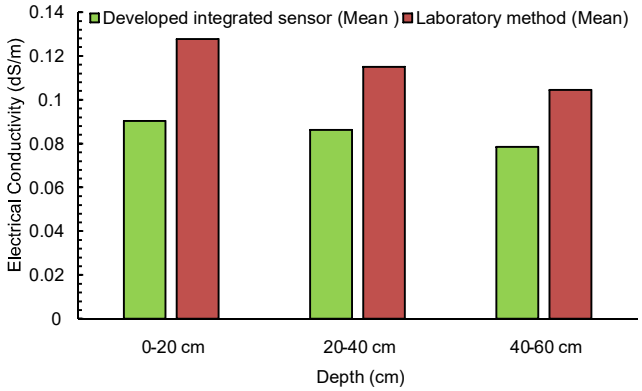

Fig. 10. Measurement of soil electrical conductivity

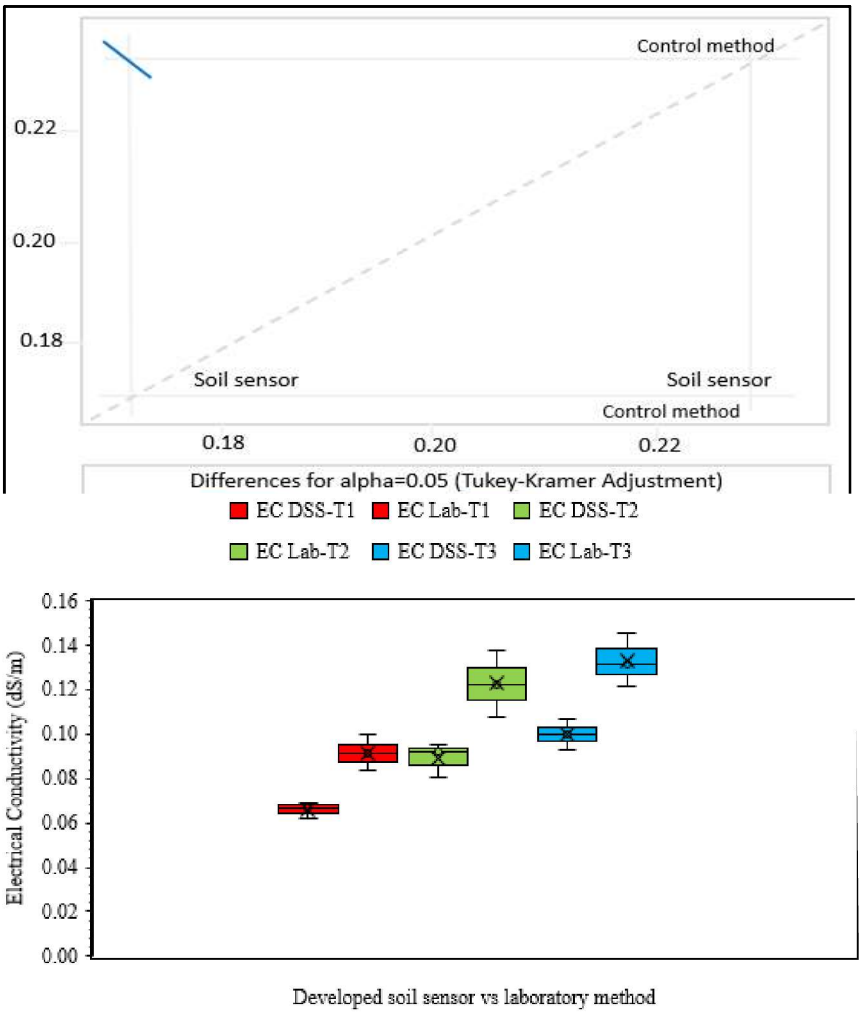

Fig. 12. Whisker's boxplot chart for soil electrical conductivity distribution by developed soil sensor (EC DSS) and laboratory method (EC Lab) in T1, T2, and T3

Table 4. Model summary of regression correlation between three different types of soil

Statistical parameters	Lab test vs measured output			
	Research farm of DFMPE, Near Gate no.4, PAU, Ludhiana	Research farm of DFMPE, Near PRSC, Gate no.6, PAU, Ludhiana	Soil research farm of DSS, Near Gate no. 8, PAU, Ludhiana	Corresponding output result of all textures
R^2 value (correlation)	0.91	0.85	0.93	0.91
R^2 value (coefficient of determination)	0.83	0.73	0.87	0.83
Adjusted R^2	0.81	0.72	0.87	0.83
Standard error of the estimate RMSE	0.0118	0.005	0.0123	0.011
Linear equation, Y	y = -0.0662x + 0.1835	y = -0.0793x + 0.1851	y = -0.0532x + 0.1775	y = -0.055x + 0.1755

CONCLUSION

The calibration of the S-type load cell, depth sensor, and electrical conductivity sensors interfaced with the microcontroller was successful, as demonstrated by the calibration results. The regression models indicated a strong correlation and a low standard error of estimate for the load cell calibration. The ultrasonic depth sensor also showed excellent calibration results, with an R^2 value of 0.99 when compared to manual distance measurements using a tape measure. Similarly, the electrical conductivity sensor exhibited a strong correlation (R^2=0.99) with laboratory EC measurement methods.

The integrated soil sensor system demonstrated an impressive accuracy exceeding 95% in measuring soil compaction during field evaluations, closely matching the results obtained through standard laboratory methods. In contrast, the assessment of soil electrical conductivity yielded an average accuracy of 75% when compared to laboratory techniques, indicating a moderate level of reliability in this measurement. Future enhancements to the accuracy of the EC sensor could be achieved through the implementation of a non-contact sensor or by redesigning the system to incorporate four probes instead of one. Overall, the results from the integrated soil sensor system confirmed that the sensors were effectively calibrated, validating the system's suitability for real-time monitoring of electrical conductivity and soil compaction, which is essential for preserving soil health.

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Spider Diversity and Abundance in Bt and Non-Bt Cotton Crops in Punjab, India

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Abstract: Bt cotton, expressing a gene for Bt-toxin specifically affects lepidopteran larvae, but little is known about its impact on spiders. This study recorded the diversity and abundance of spiders in both Bt (transgenic cotton PAU Bt 1) and non-Bt cotton (F 2228) crops along with the effect of other factors like crop stages and climate for two seasons. Spiders of nine genera and 11 species representing six families: Araneidae (37.68%), Oxyopidae (28.85%), Lycosidae (18.68%), Thomisidae (10.57%), Salticidae (3.80%), and Pisauridae (0.41%) were recorded with maximum species richness of family Araneidae followed by family Lycosidae, and families Oxyopidae, Thomisidae, Salticidae and Pisauridae. The Shannon-Weiner index and species evenness index were 1.42 and 0.59, respectively. Spider abundance was significantly higher in Bt cotton compared to non-Bt cotton indicating no adverse effect of Bt toxin on spiders. In both crops, the spider abundance was higher at the boll-bearing and boll-opening stages. Additionally, there was a significant effect on spiders of mean temperature. The study suggests that rich spider fauna of cotton crops be protected to utilize their full potential in the Integrated Insect Pest Management Program.

Keywords: Bt-toxin, Climatic factors, Cotton, *Gossypium hirsutum*, PAU Bt 1

Spiders belong to one of the most important arthropod predator groups in the world, ranking seventh in the world after six major insect orders (Mahalakshmi and Jeyaparvathi 2014). They help maintain ecosystem balance by preying on a wide variety of insects, many of which are agricultural pests, thus contributing to crop protection. The taxonomic study of spiders is advancing rapidly, with many new species discovered each year, but the world's fauna is still poorly understood. There are a total of 52,536 known spider species belonging to 134 families and 4,341 genera in the world (World Spider Catalogue 2024). Indian arachnid fauna accounts for 3.72% of the total world diversity (61 families, 493 genera, and 1947 species) (Caleb and Sankaran 2023).

Cotton is one of the most important commercial crops cultivated in India, accounting for around 23% of the total cotton production in the world. India has got first place in the world with a cotton growing area of around 40% to that of world's area of 32.42 million hectares in 2022-23. (Cotton Advisory Board 2024). Cotton crops harbour a wide variety of natural enemies, but their potential as biological control agents for insect pests has been largely overlooked in scientific research. Biological control has long been recognized as a safe, environment-friendly, and long-lasting solution for managing insect pests, but intensive agriculture and climate affect population dynamics and activities of natural enemies (Van den Berg et al., 1990).

Spiders play an important role in pest control by eating large numbers of prey animals in agricultural fields, reducing the need for chemical pesticides (Rajeswaran et al., 2005). Most spider species are omnivorous and feed on multiple life stages of prey. The abundance of spiders in crops increases during the growing stage of the plant (Mallesh and Sravanthy 2021). Despite the large number of studies on the ecology of spiders in different ecosystems worldwide, the significance of spiders as natural control agents is still largely unknown. Little attention has been paid to their insect control benefits in India because little is known about their species diversity, population abundance, and ecology.

In 2002, the Indian government decided to launch genetically modified cotton (Bollgard I cotton) called Bt cotton by inserting the gene (from soil bacterium *Bacillus thuringiensis* var. *kurstaki*) coding for Bt-toxin into cotton as a transgene, causing it to produce this natural insecticide in its tissues (Choudhary and Gaur 2010). This protects cotton crop lepidopteran pests, particularly bollworm complex (American bollworm (*Helicoverpa armigera* Hub.), spotted bollworm (*Earias vitella* Fab.), and pink bollworm (*Pectinophora gossypiella* Saunders), increasing cotton yield both quantitatively and qualitatively. Despite their relative safety in comparison to conventional insecticides and economic benefits to growers, there is still a concern that Bt crops may affect the population and diversity of upper trophic level non-target organisms, such as spiders (Head et al., 2001). The consumption of prey that survive the ingestion of Bt-toxin and thus contain traces of the toxin could indirectly affect natural enemies occurring in cotton fields (Meissle and Lang 2005). The present study aimed to record the diversity and abundance of spiders in Bt

(transgenic cotton PAU Bt 1) and non-Bt cotton crops, as well as the influence of crop stages and climate on the spider community in Punjab, India.

MATERIAL AND METHODS

The present study was undertaken in two independent lines, Bt (Variety: PAU Bt 1 containing cry 1 Ac gene) and non-Bt cotton (variety: F2228) (American cotton, *Gossypium hirsutum*: Family Malvaceae) grown in experimental plots (0.4 ha area each), at the campus of Punjab Agricultural University (PAU), Ludhiana located at an intersection of 30°55' N parallel of latitude and 75°54'E line of longitude. The crops were sown during mid-May and harvested in mid-November using agronomic practices as recommended by PAU, Ludhiana. The data was recorded in two seasons. To record the spider diversity in Bt and non-Bt cotton crops, a plot of 10m x 20m was selected in the center of each experimental crop of the Department of Entomology, PAU, Ludhiana. In each plot, spiders were searched on the plants and the soil directly under the plants and collected using a combination of hand-picking and suction methods at 15-day intervals (Ludy and Lang 2004). Large spiders were gently tapped into the collection vials, while the small spiders were collected by sucking with a homemade aspirator and then blown into a collection vial for further identification.

Immediately after collection, the spiders were counted and their various morphological features were recorded and photographed by observing them under the stereoscopic binocular microscope located in the Electron Microscopy and Nanotechnology Laboratory, PAU, Ludhiana. The spiders were then preserved in 70% ethyl alcohol for later identification. Besides, various parts of some spider specimens were also removed and placed in 10% Potassium hydroxide solution overnight to dissolve the softer parts. They were washed in water, dehydrated, and mounted on slides for detailed examination. Male spiders were identified based on swollen palpus. The male genitalia was studied in an unexpanded condition. The specimens were properly identified as per the diagnostic characteristics described (Tikader and Biswas 1981, Barrion and Litsinger 1995, Sebastian and Pete 2017). To record the abundance of spiders in Bt and non-Bt cotton crops, four plots each having an area of 10m x 20m were selected from all four geographical sides. In each plot of the crop, five plants, one each from all four geographical sides and the center of the plot were selected and marked. Visual searches were made to locate the spiders on the whole plant, from bottom to top, on both the upper and lower sides of the leaves and the soil directly under the plant for the presence of spiders. The total number of spiders present on five plants of a plot was counted to determine the average number of spiders per plot. Data was recorded at 15-day intervals on the selected plots from July to October for two seasons. Spiders found predating on insects in the crop fields were also photographed to record their predatory activity. Additionally, the data for different meteorological parameters such as mean temperature (°C), relative humidity (%), rainfall (mm), and wind velocity (km/h) were obtained from the Department of Climate Change and Agrometeorology, PAU, Ludhiana and their correlation with spider abundance was determined.

The data was analyzed using a factorial completely randomized design in SAS (Statistical Analysis System) 9.3 software. The effect of weather parameters on spider abundance was determined using a stepwise regression technique. Values were considered significant at a 5% level of significance. Shannon-Weiner index and species evenness index for spiders representing different families were calculated as per the formulae described by Nagrare et al. (2015) and are given below:

Shannon-Weiner index (H) = $\sum[(pi) \times \ln(pi)]$

Where, pi = proportion of total sample represented by species i

Species evenness (E) = $H / \ln(S)$

Where, H = Shannon-Weiner index, and

S = Total number of species

RESULTS AND DISCUSSION

Spiders of 11 species representing nine genera and six families were recorded from both Bt and non-Bt cotton crops (Table 1). A total of 523 and 451 spiders were collected from different parts of plants as well as the soil below the plant from Bt and non-Bt cotton crops, respectively. These spider species in decreasing order of their occurrence belonged to families Araneidae (37.68%) followed by Oxyopidae Lycosidae, Thomisidae, Salticidae, and Pisauridae with maximum species richness observed of family Araneidae (4) followed by family Lycosidae (3), and families Oxyopidae, Thomisidae, Salticidae and Pisauridae (1 each) in both Bt and non-Bt crops (Table 1). The relative occurrence of spiders of different families in Bt and non-Bt cotton crops was almost similar. Spider diversity was similar in both types of cotton crops with predominance of families Araneidae, Lycosidae, and Oxyopidae which together constituted 85.21% of the total spider collection. The Shannon-Weiner index and species evenness index were 1.42 and 0.59, respectively in both the crops indicating rich spider diversity but less equitable distribution. The characteristic features of these species were used for their identification (Figs. 1-4). Characteristic X-shape webs of *Neoscona theis* (family Araneidae) were also observed in the present study (Fig 1B).

Spiders were also found predating on insects in the crop fields (Fig. 5) indicating their potential as biocontrol agents.

The record of seasonal abundance of spiders in Bt cotton grown in both seasons I and II, showed a low incidence (per 200m^2 plot) of spiders in July-August (3.00-10.70) during the growth stage, followed by an increase in September-October (16.00-18.50) near boll opening stage of the crop (Table 2). Seasonal records of the spider abundance in the non-Bt cotton crop grown in the two seasons also showed the same trend with a low average population in July-August and the first fortnight of September (0.00-2.25) followed by an increase from the second fortnight of September to October (3.00-7.50) when the crop was at the boll opening stage (Table 2). Overall, spider abundance was significantly higher in Bt crops as compared to non-Bt crops indicating no adverse effect of Bt toxin on the spider population.

The stepwise regression analysis between spider abundance as the dependent variable and weather parameters, viz. mean temperature, relative humidity, rainfall, and wind velocity as independent variables revealed that spider abundance was positively correlated with the mean temperature, while relative humidity, rainfall, and wind velocity had a negative correlation with spider abundance. Further, there was a significant effect of mean temperature and rainfall on spider abundance.

In the present study, spiders of families Araneidae, Oxyopidae, and Lycosidae were most abundant on cotton crops. Bukhari et al. (2012) found spiders of the family Lycosidae (57.39%) as most abundant in the cotton crop in Gujranwala, Pakistan. Nagrare et al., (2015) observed spider diversity in transgenic and non-transgenic cotton in the rainfed agroecosystem of India and reported that the family Araneidae contributed one-third spider population (34.56%) followed by Oxyopidae (27%) and Thomisidae (24.53%). The Spiders of the family Salticidae (jumping spiders) which hunt sucking bugs like *Nezara viridula* and other hemipterans, were more common in Bt-cotton (Whitehouse et al., 2014). Wolf spiders of the family Lycosidae are abundant and

Table 1. Percent occurrence of different spiders in Bt and non-Bt cotton crops

Family	Species richness	Bt-cotton		Non-Bt cotton		Overall percent occurrence (974 total spiders)
		Number of spiders collected	Percent occurrence (%)	Number of spiders collected	Percent occurrence (%)	
Araneidae	4	205	39.20	162	35.92	37.68
Oxyopidae	1	136	26.00	145	32.15	28.85
Lycosidae	3	112	21.41	70	15.52	18.68
Thomisidae	1	57	10.90	46	10.20	10.57
Salticidae	1	10	1.91	27	5.99	3.80
Pisauridae	1	3	0.57	1	0.22	0.41
Total	11	523	--	451	--	--
H-index	--	--	1.42	--	1.42	--
Evenness	--	--	0.59	--	0.59	-

Table 2. Abundance of spider population in Bt and non-Bt cotton crops

Date	Number of spiders (n = 4 plots each)		Number of spiders (n = 4 plots each)	
	Season I		Season II	
	Bt crop	Non-Bt crop	Bt crop	Non-Bt crop
12th July	3.00±1.58^{A}	0.75±0.83^{B}	6.50±2.61^{A}	1.25±0.43^{B}
27th July	5.75±4.21^{A}	1.75±1.30^{B}	7.00±1.41^{A}	1.00±1.22^{B}
12th August	4.25±2.38^{A}	0.00±0.00^{B}	10.70±3.34^{A}	1.00±1.73^{B}
27th August	6.75±1.48^{A}	1.25±0.43^{B}	9.00±3.93^{A}	1.75±1.30^{B}
12th September	16.25±2.05^{A}	2.25±1.64^{B}	18.50±1.50^{A}	1.50±1.12^{B}
27th September	16.00±2.83^{A}	4.75±2.80^{B}	16.70±3.70^{A}	3.00±2.54^{B}
12th October	16.50±1.91^{A}	7.25±3.96^{B}	10.70±3.27^{A}	4.25±2.38^{B}
27th October	17.50±2.52^{A}	7.50±3.64^{B}	8.50±2.17^{A}	3.75±1.48^{B}

Values are Mean±SD, Values with superscripts (A,B) in a row for two seasons, separately indicate significant differences at P<0.05

rapacious predators at the soil-plant interface in cotton crops. They prey on late-instar larvae of economically important cotton bollworm *Helicoverpa* spp., that pupate in the soil (Rendon et al., 2019).

In the present study, the Shannon-Weiner index (1.42) and species evenness index (0.59) were low in both Bt and non-Bt cotton crops. Bukhari et al. (2012) have reported a higher Shannon-Weiner diversity index (2.80) and species evenness (0.92) for spider species collected from cotton crops in Pakistan. Nagrare et al. (2015) reported that the Shannon-Weiner index was 1.64 and the evenness index was 0.92 for spiders in transgenic and non-transgenic cotton crops in Central India. In the present study in both Bt and non-Bt cotton crops, spider abundance was generally low during the growth period of the crops i.e. in July-August and high near boll bearing and boll opening stages in September-October which are the times when most of the insects visit the crop and cause damage to the bolls and the plant has maximum height and foliage. This may be the reason for the buildup in the number of spiders and other natural enemies (Rajendran et al., 2018). Bt cotton also acts as a refuge for spiders and other insect predators which get sufficient food available in the field from these non-lepidopteran insects (Mehboob-ur-Rahman and Zafar 2018). This may be the reason for the significantly higher spider abundance observed in Bt crops in the present study.

Bt cotton has no adverse impact on spider abundance and distribution of natural insect predators under field conditions (Dhillon et al., 2012, Arshad et al., 2015) although community composition could differ due to differences in the pest complex (Whitehouse et al., 2014). In the present study also, a higher number of spiders were collected from Bt crops (523) as compared to non-Bt crops (451). Contrary to the present results, Kiranmai and Sammaiah (2018) found a higher abundance of arthropods (including spiders) in non-Bt cotton compared to Bt cotton fields. The lower spider population in July-August during the present study may also be due to heavy rainfall. A significant negative effect of rainfall was observed on spider abundance in the present study. The study showed that spider population was positively

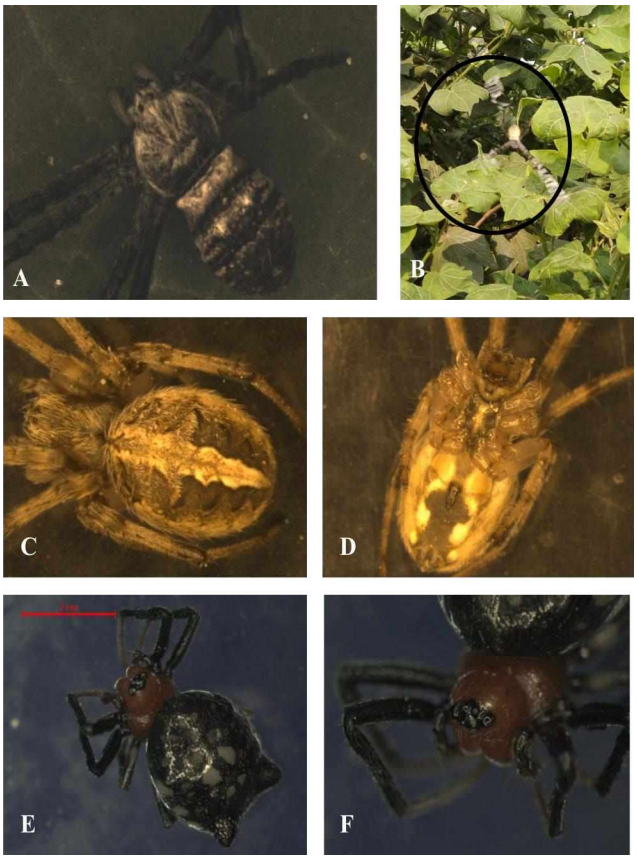

Fig. 1. *Argiope aemula*, A) Dorsal view and B) Characteristic X-shapes web; *Neoscona theis,* C) Dorsal view and D) Ventral view showing epigynum; *Chorizpoes bengalensis*, E) Dorsal view, and F) Front view showing eye pattern

Fig. 2. *Neoscona mukerji*, A) Dorsal view and B) Ventral view; *Plexippus paykulli,* C) Dorsal view and D) Front view showing eye pattern; *Thomisus lobosus,* E) Dorsal view, and F) Ventral view.

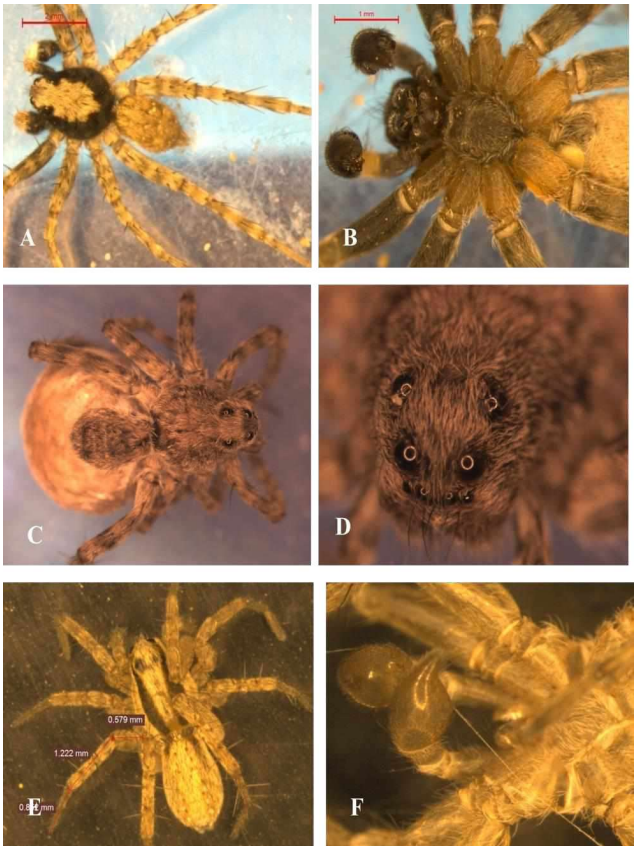

Fig. 3. *Lycosa mackenziei*, A) Dorsal view and B) Ventral view showing sternum and shape of male palps; *Pardosa pseudoannulata,* C) Dorsal view with egg case attached to abdomen and D) Front view showing eye pattern; *Pardosa sumatrana*, E) Dorsal view, and F) Anterior view showing shape of male palps

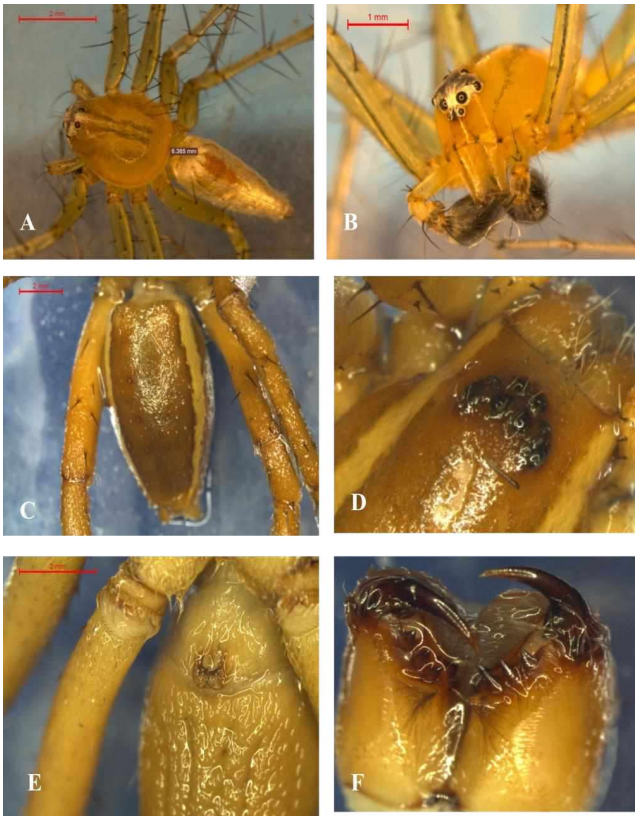

Fig. 4. *Oxyopes birmanicus*, A) Dorsal view and B) Front view showing eye pattern; *Perenethis venusta,* C) Dorsal view of abdomen, D) Dorsal view of cephalothorax, E) Ventral view of abdomen showing epigynum, and F) Chelicerae showing arrangement of fangs and teeth

correlated with mean temperature, while relative humidity, rainfall, and wind velocity were negatively correlated. Similar to our results, Bukhari et al. (2012) reported that increasing rainfall and high relative humidity suppressed spider populations in July and August in the cotton crop of District Faisalabad, Pakistan. Khuhro et al. (2012) reported a negative effect of both temperature and relative humidity on the spider population in cotton crops, while, Muchhadiya et al. (2014) reported a significant positive association of spiders with rainfall.

CONCLUSIONS

There was no significant effect of Bt toxin on spider diversity and abundance in cotton crops. The abundance of spider communities, was, however, affected by climatic factors and the stage of the crop. There was a rich spider fauna in Bt and non-Bt cotton crops, and evidence of spiders predating on insects suggests utilization of their full potential in the Integrated Pest Managment program.

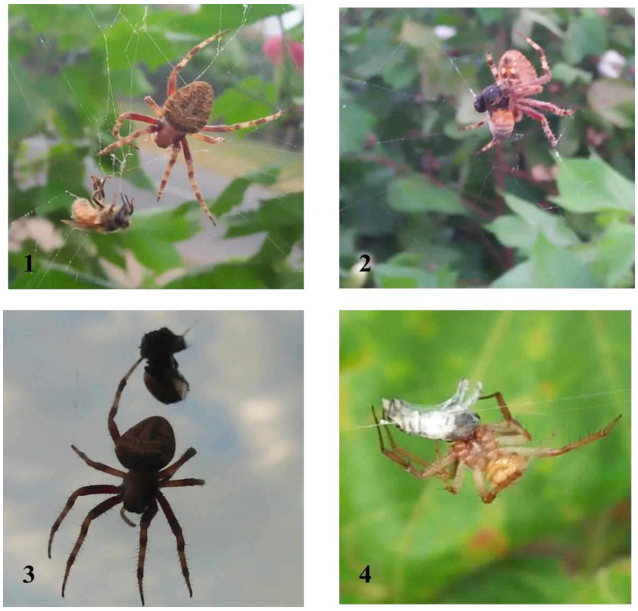

Fig. 5. Spiders found predating on different insects in cotton fields, 1) Honey bee, *Apis mellifera,* 2) Scoliid wasp, *Scolia erythropyga,* 3) lady bird beetle, *Coccinella septempunctata,* and 4) Cotton grey weevil, *Chorthippus albomarginatus*

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Diversity, Abundance and Diurnal Activity of Insect Pollinators on Onion (*Allium cepa*) in Northern Transitional Zone of Karnataka

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***Abstract*:** The study on insect pollinators of onion (*Allium cepa*) recorded 16 species from 11 families across four orders, with hymenopterans being the dominant group (94.2%), followed by Diptera (3.1%), Lepidoptera (1.4%), and Coleoptera (1.3%). Among honeybees, *Apis florea* was the most abundant pollinator (34.65%), followed by *Tetragonula* sp. (30.84%). Pollinator activity peaked at 1000-1100 h, increasing with flowering progression from 50% to 90%. Maximum activity (7.73 visitors/m²/5 min) occurred at 90% flowering. Diversity indices indicated higher species richness at 90% flowering (Shannon-Wiener index: 1.81; Simpson diversity: 0.81) compared to 50% flowering (Shannon-Wiener index: 1.67; Simpson diversity: 0.77). The findings highlight the significance of hymenopterans as primary pollinators. This study emphasizes the critical role of diverse insect pollinators in onion crop productivity, with peak pollination activity occurring during mid-morning hours.

***Keywords*:** Diversity, Hymenopterans, Onion, Pollinator activity

Onion, *Allium cepa* L., is a well-known bulbous vegetable from Central Asia and part of the Liliaceae family. For over 5000 years. In terms of onion bulb production, India ranks second after China, producing 311.29 lakh tonnes with a productivity rate of 16.26 MT/ha (Anonymous 2022). Despite the high production levels, the average yield of onions in India falls below the global standard. This disparity is primarily due to the lack of quality seed material. India requires 9400 tonnes of onion seeds each year to cultivate the 11.73 lakh ha area. About 40% of the onion seed demand is fulfilled by the organized sector, while farmers supply the remaining. The quality of onion seeds is closely linked to pollination, which depends on the number and variety of insect pollinators. Hence, it is crucial to protect these insect visitors to ensure the production of high-quality and abundant onion seeds.

Onion is a crop that experiences a high degree of cross-pollination due to its protandrous characteristics (Kavitha and Rami Reddy 2018). Self-pollination is uncommon, and the plant depends on insects for cross-pollination (Karuppiah et al., 2017). The umbelliferous inflorescence can be seen at the apex of the hollow green stalk (Kavitha and Rami Reddy 2018). Various factors influence the number of insect visitors to onion flowers, including their size, shape and colour, along with environmental conditions and the availability of floral rewards. The pollen and nectar produced by onion blooms attract a range of insect visitors, such as honey bees, syrphid flies, drone flies, halictid bees, butterflies, and bumblebees (Sajjad et al., 2008). Various pollinators have been identified as significant contributors in different ecological regions around the globe. Stingless bees (Karuppaiah et al., 2017) and honey bees of the genus *Apis*, namely *A. dorsata* (Devi et al., 2014, Saeed et al., 2008), *A. cerana indica* (Hosamani et al., 2019), *A. mellifera* (Mazeed et al., 2018) and *A. florea* (Saeed et al., 2008), along with syrphids (Chandel et al., 2004) are noted for their effectiveness as pollinators in the onion ecosystem due to their body size, morphology, and short tongue (Pusphpalatha et al., 2023). Onion plants fail to produce quality seeds without insect visitation to their flowers. Research has been carried out to assess the abundance of various natural pollinators and the impact of bee pollination on the quantity and quality of onion seeds. In India, the population of natural insect pollinators is rapidly declining due to the ongoing use of pesticides and the loss of natural habitats. For instance, in crops like sunflowers, there is a 30 per cent reduction in flower-visiting insects (Chaudhary and Poonia 2018). The current study examined the diversity of different insect pollinators on onion crop within the Dharwad region of Karnataka.

MATERIAL AND METHODS

The present investigations were carried out in the Saidapur farm, University of Agricultural Sciences, Dharwad (15°26' North latitude, 75°07' East longitudes and at an altitude of 678 meters above mean sea level) during *Rabi* 2021-22. The experimental area is located in the Northern transitional zone (zone VIII) of Karnataka, which receives

700-800 mm average annual rainfall. The temperature and relative humidity range from 12-37 °C and 40 to 85 per cent respectively.

The bulbs of the onion variety 'Bhima super' were planted on 10th November 2021. The crop came to bloom by the end of February 2022. The experimental plot was raised as per the package of practices except for plant protection measures during the flowering period. Different species of insect pollinators visiting the umbels of the onion crop were observed. The visual count was done in onion under open pollination conditions. Randomly, five spots of one square meter area were selected and observed for 2 minutes at different time intervals of 0800-0900 h, 1000- 1100 h, 1300-1400 h and 1600-1700 h at 50, 75 and 90 per cent flowering. During the period of observation, Samples collected were placed in ethyl acetate as the killing agent, pinned, preserved and identified by the taxonomical expert of the Division of Entomology, Dr. Yeshwanth, GKVK, Bangalore.

The relative abundance of pollinators was calculated.

$$\text{Relative abundance} = \frac{\text{Abundance of the species}}{\text{Total abundance of all species}} \times 100$$

Shannon-Wiener diversity index (H):

$H = -\Sigma\, pi \ln pi$

Where,

pi = Proportion of the i^{th} species of pollinator

ln = Natural log with base e=2.718

Simpson index of diversity (1-D): $D = 1-\Sigma pi$

RESULTS AND DISCUSSION

The insect pollinators on onion include 16 species belonging to 11 families of 4 orders. The hymenopterans were the most abundant insect pollinators (94.20%) followed by Diptera, Lepidoptera and Coleoptera. The relative abundance of *Apis florea* was highest (34.65%) followed by *Tetragonula* sp. *A. cerana indica* and *A. dorsata* . Among the Dipterans, two syrphid species, *Phytomia errans* (2.64%) and *Ischidon scutellaris* (0.43 %) were recorded. Other insects *Chrysomia bezziana Oplodontha viridula, Scolia* sp., *Delta* sp., *Luthrodes pandava* (Horsfield), *Danaus chrysippus, Euchromia polymena, Aulacophora foveicollis, Coccinella transversalis* and *Cheilomenes sexmaculata* (Plate 1, 2) activity was frequent and rare during the cropping season (Table 1).

Onion flowers are rich in pollen and nectar content which attracted the pollinators. While hopping from plant to plant in quest of their prey, coccinellid species may accidentally act as pollinators. Lepidopterans that feed on nectar while visiting flowers regularly also serve as pollinators. The pollinator fauna of onion has been well documented by several workers in different parts of the country. Basavaraj (2004) reported that Hymenopteran pollinators accounted for the majority (90.15%) followed by Diptera (6.63%) Lepidoptera (1.70%) and other pollinators (1.52%) in onion. TAbrol (2010) identified *A. florea* as the key pollinator species. Hymenopterans comprised 60 per cent of pollinators followed by Diptera, Lepidoptera and Coleoptera (Devi et al., 2014). Karuppaiah et al. (2018) revealed that onion was pollinated by eleven different insect species where Hymenopterans contributed 98 per cent. Hosamani et al. (2019) observed that most of the pollinators on onion were Hymenopterans (87.79%), followed by Dipterans, and Lepidopterans. Four species of honeybees from the Apidae family made up the majority of pollinators that visited the

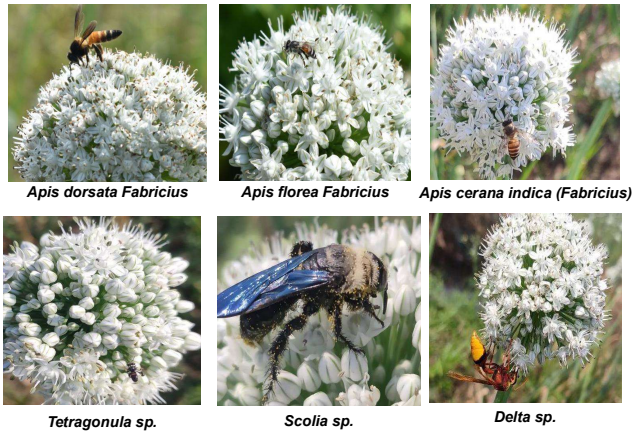

Plate 1. Hymenopteran pollinators

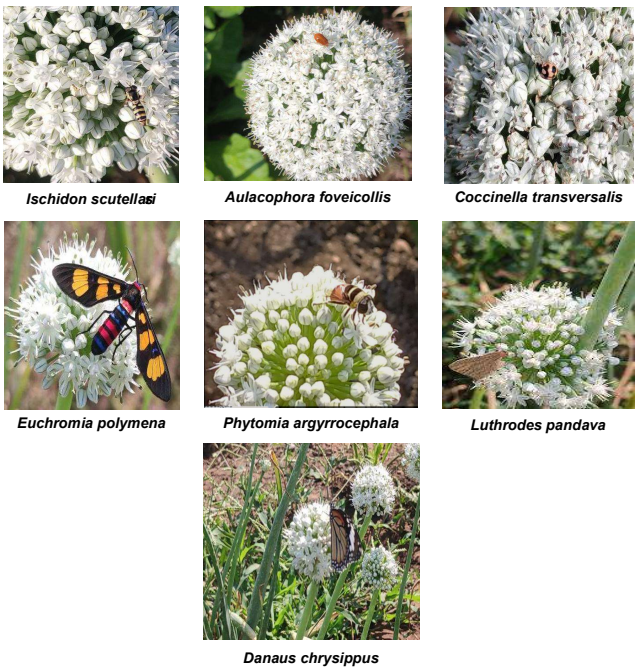

Plate 2. Pollinators recorded on onion

onion crop. Maragoor et al. (2022) further validated that *A. florea* is the primary pollinator, followed by *A. cerana indica* and syrphids in the coriander and ajwain ecosystems of Northern Karnataka.

Pollinator activity peaked at 1000-1100 h (4.78 visitors/m^2/5 min) and lowest at 0800-0900 h (1.80 visitors/m^2/5 min) at 50 per cent flowering under open pollination conditions. Pollinator activity was moderate between 1300-1400 h (3.63 visitors/m^2/5 min) and 1600-1700 h (2.78 visitors/m^2/5 min). The most prevalent pollinators that were active across all observational periods were Hymenopterans. Between 1000-1100 h, *Tetragonula* sp. (7.75 visitors/m^2/5 min) and *A. florea* (8.75 visitors/m^2/5 min) were the two most active pollinators, while lepidopteran activity was minimal ranging between 0.00 and 0.4 visitors/m^2/5 min (Table 2). At 75 per cent flowering, pollinator activity was maximum (6.2 visitors/m^2/5 min) between 1000-1100 h and was lowest (2.3 visitors/m^2/5) at 0800-0900 h. It was moderate between 1300-1400 h (4.93 visitors/m^2/5 min) and 1600-1700 h (3.63 visitors/m^2/5 min). Hymenopterans were the most common pollinators active at all intervals observed. Maximum number of *A. florea* and *Tetragonula* sp., was recorded from 1000 to 1100 hours (15.4, 13.4, visitors/m^2/5min respectively). Among these, *A. florea* was the dominant pollinator (11.1 visitors/m^2/5 min), followed by *Tetragonula* sp. (9.15 visitors/m^2/5 min), while

Table 1. Diversity of insect pollinators on onion

Common name	Scientific name	Family	Order	Species abundance (%)	Relative abundance % (Order)
Little bee	*Apis florea*	Apidae	Hymenoptera	34.65	94.20
Indian bee	*A. cerana indica*			15.11	
Rock bee	*A. dorsata*			13.10	
Stingless bee	*Tetragonula* sp.			30.84	
Scoliid wasp	*Scolia sp.*	Scoliidae		0.47	
Potter wasp	*Delta sp.*	Vespidae		0.03	
Syrphid	*Phytomia errans*	Syrphidae	Diptera	2.64	3.10
Common hoverfly	*Ischidon scutellaris*			0.43	
Common green colonel	*Oplodontha viridula*	Stratiomyidae		0.02	
Blow fly	*Chrysomia sp.*	Calliphoridae		0.01	
Plain cupid	*Luthrodes pandava* (Horsfield)	Lycaenidae	Lepidoptera		1.40
Plain Tiger Butterfly	*Danaus Chrysippus*	Nymphalidae			
Wasp moth	*Euchromia polymena*	Erebidae			
Red pumpkin beetle	*Aulacophora foveicollis*	Chrysomelidae	Coleoptera		1.30
Transverse ladybird	*Coccinella transversalis*	Coccinellidae			
Indian wave striped ladybug	*Cheilomenes sexmaculata*				

Table 2. Diurnal variation in activity of pollinators at different hours at 50 per cent flowering in onion

Species	Number of visitors/m^2/5 minutes					
	08.00-09.00 h	10.00-11.00 h	13.00-14.00 h	16.00-17.00 h	Total	Mean±SD
A. florea	5.4	12.6	9.4	7.6	35	8.75 ±3.04
A. cerana indica	2.0	5.0	3.4	2.8	13.2	3.3±1.27
A. dorsata	1.6	5.6	4.2	3.6	15	3.75 ±1.66
Tetragonula sp.	4.6	11.2	9.0	6.2	31	7.75 ± 2.93
P. errans	0.4	1.6	1.2	0.8	4	1±0.52
I. scutellaris	0.2	1.4	1.0	0.6	3.2	0.8±0.52
Lepidopterans	0.0	0.4	0.2	0.4	1	0.25±0.19
Others	0.2	0.4	0.6	0.2	1.4	0.35±0.19
Total	14.4	38.2	29	22.2	103.8	26±10.11
Mean	1.80	4.78	3.63	2.78	12.97	3.24

other pollinators activity was low (Table 3). Activity of pollinators at 90 per cent flowering was maximum between 1000-1100 h (7.73 visitors/m^2/5 min) and minimum between 0800-0900 h (3.6 visitors/m^2/5 min), while it was moderate between 1300-1400 h (6.3 visitors/m^2/5 min) and 1600-1700 h (4.7 visitors/m^2/5 min). Hymenopterans were the most predominant pollinators, which were active throughout the day. However, the maximum number of *A. florea* and *Tetragonula* sp was during 1000-1100 h (18.6, 16.8, visitors/m^2/5 min respectively). *A. florea* was the dominant pollinator (14.00 mean visitors/m^2/5 min), followed by *Tetragonula* sp. (12.3 visitors/m^2/5min), while the activity of other pollinators was less and ranged from 0.2 to 0.8 visitors/m^2/5min (Table 4). As there is an increase in flowering from 50 per cent towards 90 per cent, the activity of pollinators also increased in onion. Within a day, the activity of most of the pollinating species was maximum at 1000-1100 h (Fig.1). Hymenopterans were the most common pollinators, which were active at all observation intervals. Among the honeybees, *A. florea* was the most predominant pollinator, followed by *Tetragonula* sp. and peak activity was d during 1000-1100 h. The current findings are in accordance with the previous findings. The rate of pollinator visitation was notably high, peaking at 1000 hours for onion, aligning with observations made by Saleh et al. (2021). The maximum abundance of *A. florea* was recorded between 1200 and 1400 hours. In this study, the highest visitation rates for onions were observed during the intervals of 1000-1100 hours and subsequently from 1300-1400 hours, likely influenced by variations in climatic conditions. Conversely, in coriander, peak visitation was noted during the 1000-1100 hours interval, as documented by Bhowmik et al. (2017), and another report indicated highest visitation between 1000 and 1200 hours in coriander (Usman et al., 2018) and onion (Biradar et al., 2017).

Among the different flowering stages (50, 75 and 90 %),

Table 3. Diurnal variation in activity of pollinators at different hours at 75 per cent flowering in onion

Species	Number of visitors/m^2/5 minutes					
	08.00-09.00 h	10.00-11.00 h	13.00-14.00 h	16.00-17.00 h	Total	Mean±SD
A. florea	6.2	15.4	12.8	10.0	44.4	11.1±3.94
A. cerana indica	2.8	7.2	5.6	4.0	19.6	4.9±1.91
A. dorsata	2.0	7.0	5.2	3.6	17.8	4.45±2.14
Tetragonula sp.	5.2	13.4	10.6	7.4	36.6	9.15±3.59
P. errans	1.0	2.8	2.2	1.6	7.6	1.9±0.77
I. scutellaris	0.6	2.6	2.0	1.2	6.4	1.6±0.87
Lepidopterans	0.2	0.6	0.4	0.8	2	0.5±0.26
Others	0.2	0.6	0.6	0.4	1.8	0.45±0.19
Total	18.2	49.6	39.4	29	136.2	34.05±13.50
Mean	2.3	6.2	4.93	3.63	17.03	4.26±1.67

Table 4. Diurnal variation in activity of pollinators at different hours at 90 per cent flowering in onion

Species	Number of visitors/m^2/5 minutes					
	08.00-09.00 h	10.00-11.00 h	13.00-14.00 h	16.00-17.00 h	Total	Mean±SD
A. florea	9.4	18.6	16.8	11.2	56	14±4.39
A. cerana indica	5.4	9.8	7.2	6.0	28.4	7.1±1.95
A. dorsata	2.6	8.6	6.0	4.8	22	5.5±2.50
Tetragonula sp.	7.6	16.8	15.0	9.8	49.2	12.3±4.31
P. errans	1.8	3.2	2.8	2.2	10	2.5±0.62
I. scutellaris	1.4	3.0	2.6	2.0	9	2.25±0.7
Lepidopterans	0.4	1	0.6	0.8	2.8	0.7±0.26
Others	0.2	0.8	0.4	0.8	2.2	0.55±0.3
Total	28.8	61.8	51.4	37.6	179.6	44.9±14.61
Mean	3.6	7.73	6.43	4.7	22.45	5.61±1.83

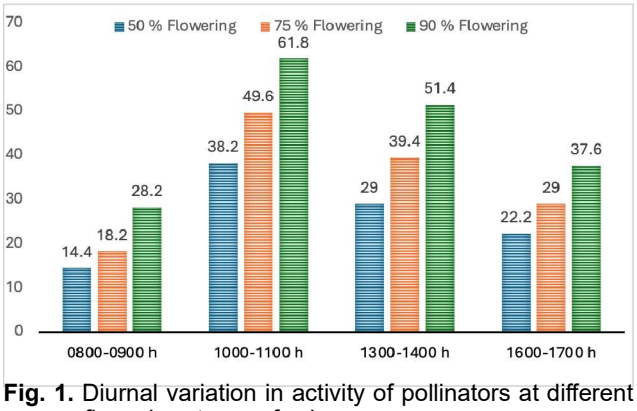

Fig. 1. Diurnal variation in activity of pollinators at different flowering stages of onion

Table 5. Diversity indices of pollinators at different flowering stages in onion

Stages of flowering		
50 %	75 %	90 %
Shannon-Wiener index (H)		
1.67	1.78	1.81
Simpson index of diversity (1-D)		
0.77	0.79	0.81

diversity indices (H and 1-D) of pollinators in onion were higher during 90 per cent flowering, followed by 75 per cent and 50 per cent flowering. The Shannon wiener index (H) ranged from 1.67 t to 1.81 at 50% and 90% flowering. Simpson index of diversity (1-D) ranged from 0.77 at 50 per cent flowering to 0.81 at 90 per cent flowering (Table 5). At 50 per cent flowering, the Shannon wiener index (H) was lowest (1.67) and highest (1.81) at 90 per cent flowering. Simpson index of diversity (1-D) was lowest (0.77) at 50 per cent and maximum (0.81) at 90 per cent flowering. (Table 5). The diversity indices (H and 1-D) of insect pollinators in onion were higher at 90 per cent flowering, followed by 75 and 50 per cent because the availability of pollen and nectar content increased towards peak flowering following an increase in the density and diversity of pollinators. Shannon-Weiner index of 1.805 in onion was reported by Karuppaiah et al. (2018), which supports the present findings.

CONCLUSION

The Hymenopterans, especially *Apis florea* and *Tetragonula* sp., are the primary pollinators for onion crop in Dharwad region. Pollinator activity reaches its highest point between 10:00 and 11:00 AM, and increases as the flowers develop from 50% to 90% blooming, with the greatest diversity indices recorded at the 90% flowering stage. These results emphasize the vital role of insect pollinators, particularly honeybees, in onion cultivation.

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AUTHOR'S CONTRIBUTION

CA performed the experiment, captured and prepared the original manuscript. KDN conceptualised the research SBK and HSM reviewed and corrected the manuscript. RNM analysed the data.

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Species Richness and Diversity of Insect Pollinators Associated with Agro-ecosystems in Kumaun Hills of Nainital District, Uttarakhand, India

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Abstract: The present study investigates the diversity of pollinating insects in the Kumaun hills of Nainital District, Uttarakhand, India, with a specific focus on the Paharpani region. The study is dedicated to investigating the diversity and abundance of insect pollinators in orchards and agricultural land in the Kumaun Himalaya of Uttarakhand. The study was conducted from March 2019 to February 2021, and total of 77 insects belonging to 7 orders, including Lepidoptera, Hymenoptera, Coleoptera, Diptera, Orthoptera, Hemiptera, and Thysanoptera, were collected from study sites. The maximum number of species belonged to the order Lepidoptera; the lowest number was found in Thysanoptera from the study site. Species richness was highest during the rainy (10.37), summer (8.979) seasons and lower in the winter (5.013).

Keywords: Diversity, Pollinators, Diversity indices, Agricultural ecosystems, Kumaun hills

Pollination is a crucial process for the reproduction of most flowering plant species and is ultimately necessary for the production of food (Nunes-Silva et al., 2010). Fruit, vegetable, or seed production from 87 of the leading global food crops depends on animal pollination (Klein et al., 2007). Insects are responsible for over 80 percent of pollination activity. Insects, including bees, are crucial for the pollination of many cultivated and wild plants (Thapa 2006). Different crops have varying pollination needs, which affects their reliance on insect pollinators (Morse and Calderone 2000). The presence of pollinators provides a unique method for monitoring ecosystem health. Various insect species from different orders play crucial roles as pollinators/visitors in the pollination of different crops (Mitra et al., 2008, Divija et al., 2022). Various insect groups that are significantly important for the pollination of agricultural, horticultural, and medicinal herbal crops mainly belong to the Hymenoptera, Diptera, Coleoptera, Lepidoptera, Thysanoptera, Hemiptera, and Neuroptera (Free 1993, Kearns et al., 1998, Mitra and Parui 2002, Mitra et al., 2008). The current study presents crucial aspects of the diversity, richness, and abundance of insect pollinators.

MATERIAL AND METHODS

Study Area: The present study was conducted in agroecosystems located at Paharpani (2044 masl, 29° 25'38N, 79° 42'41E), in Nainital district of Uttarakhand (Fig. 1, 2) from March 2019 to February 2021. In Paharpani, *Brassica oleracea*, *Brassica sinapis*, *Coriandrum sativum*, *Solanum tuberosum*, *Cucumis sativus*, *Pisum sativum*, *Allium cepa*, *Raphanus sativus*, *Phaseolus vulgaris*, *Capsicum annuum*. In orchards, *Citrus limon*, *Citrus sinensis*, *Prunus domestica*, *Malus domesticus*, *Pyrus persica* and *Pyrus communis* are grown.

Sampling and collecting of insects: The site was regularly visited every month between 9:00 am and 2:00 pm when the insects were most active. Insects were sampled at 30-day intervals using sweeping and hand-picking. After collection, the insects were placed in jars with ethyl acetate-soaked cotton and taken to the laboratory for processing and preservation in wooden boxes. The insects were identified using available literature and identification keys, and they were sorted into different orders, families, and species. Representative species were preserved in the laboratory, while unidentified ones were sent to the Zoological Survey of India in Dehradun for further identification. The trophic level in the food chain was assigned to the insects as Phytophagous, predators, polyphagous, nectarivorous, parasitoids, florivorous, saprophagous, polyelectic, and detritivorous (Table 1). Shannon's diversity index (Shannon-Wiener diversity 1949), evenness (Hill 1973), and Margalef's species richness index (Margalef's 1970) were calculated.

RESULTS AND DISCUSSION

The total of 77 species were collected from the study site. The highest number of species belonged to the order Lepidoptera (35), followed by Hymenoptera , Coleoptera , Diptera. Lepidoptera, Hymenoptera, Coleoptera, and Diptera had high abundance and species richness, while Orthoptera, Hemiptera, and Thysanoptera were minor constituents (Table 1). Species richness was highest during the rainy

(10.37), summer (8.979) seasons and lower in the winter (5.013) (Table 3). Insect abundance was significantly correlated with maximum temperature (Fig. 4a), minimum temperature (Fig. 4b), and rainfall (Fig. 4c). Both low and high temperatures, as well as rainfall, impacted the species richness and abundance of insect pollinators (Fig. 5). These findings align with previous studies (Dev et al., 2009, Regniere et al., 2012, Nadia et al., 2015, Abbas et al., 2014, Rekha 2021).

Nine trophic groups were identified: phytophagous, predators, polyphagous, nectarivorous, parasitoids, florivorous, saprophagous, polyelectic, and detritivorous. Phytophagous insects were predominant, comprising 54.05% of species and 42.28% of individuals. Predators followed at 18.68% of species and 26.93% of individuals, with polyphagous insects at 10.08% of species and 15.36% of individuals. The other groups made up smaller percentages (Fig. 3). Many ecologists have grouped insects into functional trophic guilds to study their ecological interactions (Speight et al., 2008). These findings align with global trends,

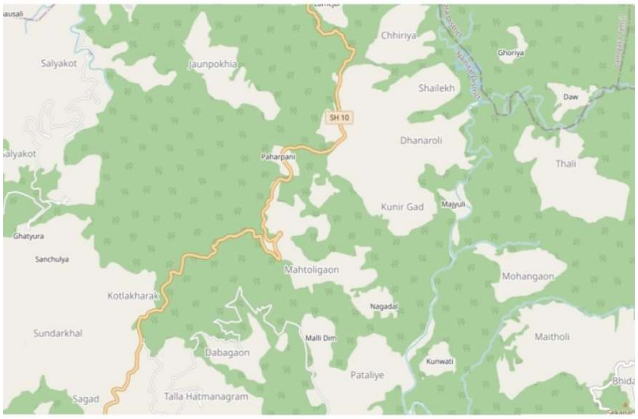

Fig. 1. Map of study area

Fig. 2. General view of study area

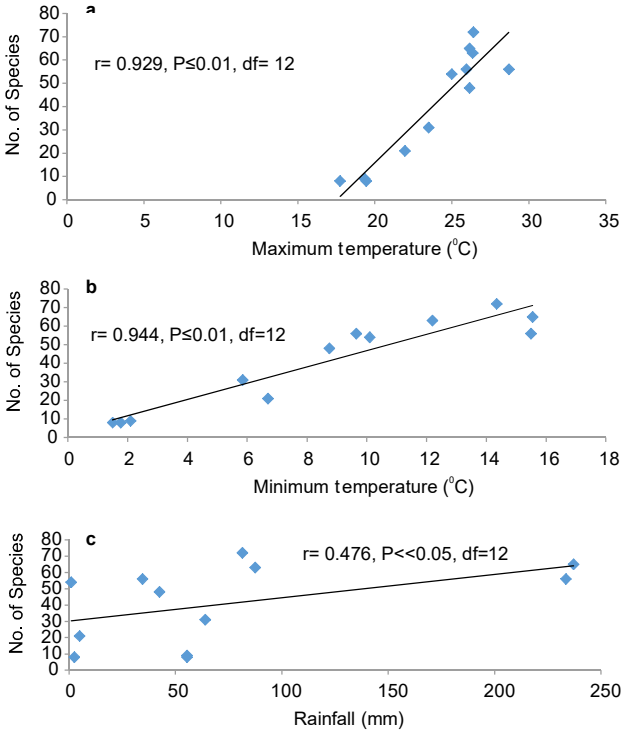

Fig. 4a-c. Correlation between Species richness with minimum temperature, maximum temperature and rainfall during March, 2019 to February, 2021

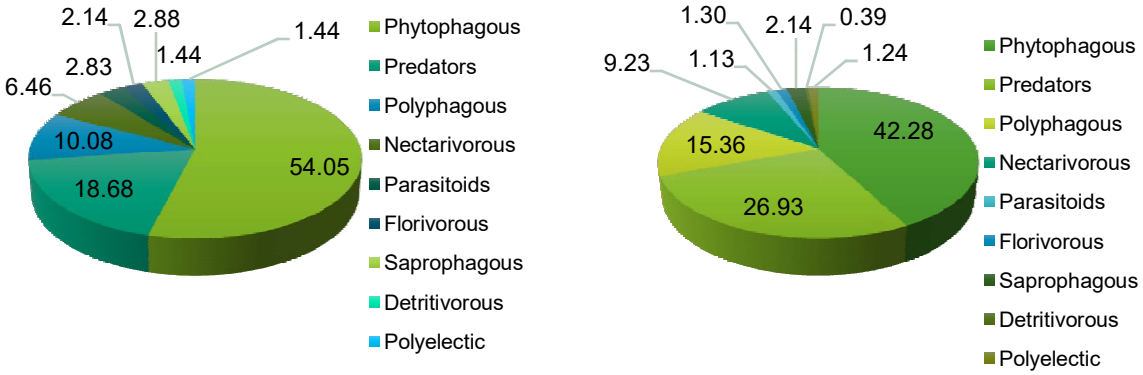

Fig. 3. Guild structure of insects fauna (species individuals)

Table 1. Diversity and relative abundance (%) of insect pollinators and trophic components during March, 2019 to February, 2021

Taxonomic composition	Trophic level	2019-2020		2020-2021	
		No. of individuals	Relative abundance (%)	No. of individuals	Relative abundance (%)
ORDER- LEPIDOPTERA					
Family: Pieridae					
Pieris canidia indica (Evans)	Phytophagous	31	1.95	39	2.65
P. brassicae (Linnaeus)	Phytophagous	28	1.76	23	1.56
Pontia daplidice (Linnaeus)	Phytophagous	21	1.32	17	1.16
Goneptyryx rhamni nepalensis (Doubleday)	Phytophagous	19	1.19	15	1.02
Aporia agathon (Gray)	Phytophagous	17	1.07	08	0.54
Colias felidi (Menetries)	Phytophagous	22	1.38	16	1.09
Catopsilia pyranthe (Linnaeus)	Phytophagous	00	0.00	17	1.16
Family: Nymphalidae					
Aglais cashmirensis (Kollar)	Phytophagous	23	1.44	33	2.24
Vanessa cardui (Herbst)	Phytophagous	31	1.95	23	1.56
Danaus chrysippus (Linnaeus)	Phytophagous	00	0.00	16	1.09
Callerebia scanda Kollar	Phytophagous	07	0.44	05	0.34
C. nirmala Moore	Phytophagous	10	0.63	08	0.54
C. annada (Moore)	Phytophagous	13	0.82	10	0.68
C. hybrida Butler	Phytophagous	11	0.69	13	0.88
Ypthima inica Hewiston	Phytophagous	15	0.94	07	0.48
Acraea issoria (Hubner)	Phytophagous	20	1.26	16	1.09
Cyrestis thyodamas Boisduval	Phytophagous	12	0.75	09	0.61
Lasiommata schakra Kollar	Phytophagous	15	0.94	12	0.82
Junonia iphita (Cramer)	Phytophagous	00	0.00	06	0.41
Family: Lycaenidae					
Lycaena pavana Kollar	Phytophagous	29	1.82	36	2.45
L. phlaeas (Linnaeus)	Phytophagous	27	1.70	20	1.36
Zizzeria sp.	Phytophagous	36	2.26	32	2.18
Heliophorus androcles (Hewitson)	Polyphagous	30	1.88	19	1.29
H. sena (Kollar)	Phytophagous	00	0.00	23	1.56
Euchrysops cnejus Fabricius	Polyphagous	26	1.63	19	1.29
Zizina otis Fabricius	Polyphagous	29	1.82	24	1.63
Pseudozizeeria maha Kollar	Polyphagous	24	1.51	32	2.18
Dodona durga (Kollar)	Phytophagous	18	1.13	25	1.70
Aricia agestis (Bergstrasser)	Phytophagous	20	1.26	21	1.43
Acytolepis puspa (Horsfield)	Phytophagous	31	1.95	22	1.50
Telicada nyseus Guerin-Meneville	Phytophagous	18	1.13	17	1.16
Family: Papilionidae					
Papilio polytes Linnaeus	Phytophagous	20	1.26	18	1.22
Atrophanura polyeuctus (Doubleday)	Phytophagous	00	0.00	12	0.82
Family: Hespiriidae					
Borbo bevani Moore	Phytophagous	07	0.44	00	0.00
Family: Sphingidae					
Rhopalopsyche nycteris Kollar	Phytophagous	02	0.13	00	0.00

Cont...

Table 1. Diversity and relative abundance (%) of insect pollinators and trophic components during March, 2019 to February, 2021

Taxonomic composition	Trophic level	2019-2020		2020-2021	
		No. of individuals	Relative abundance (%)	No. of individuals	Relative abundance (%)
ORDER- HYMENOPTERA Family: Apidae					
Apis cerena indica Fabricius	Nectarivorous	65	4.08	62	4.22
A. mellifera Linnaeus	Nectarivorous	48	3.02	44	2.99
A. dorsata Fabricius	Nectarivorous	29	1.82	21	1.43
Bombus haemarrhoidalis Smith	Phytophagous	08	0.50	05	0.34
B. festivus Smith	Phytophagous	05	0.31	08	0.54
B. hypnorum Linnaeus	Phytophagous	11	0.69	00	0.00
B. ternarius Say	Phytophagous	09	0.57	11	0.75
Ceratina sp.	Nectarivorous	08	0.50	05	0.34
Family: Halictidae					
Nomia sp.	Florivorous	05	0.25	00	0.00
Family: Xylocopidae					
Xylocopa sp.	Phytophagous	16	0.81	10	0.68
Family: Vespidae					
Vespa auraria Smith	Predator	05	0.66	09	0.61
V. basalis Smith	Predator	06	0.56	00	0.00
V. mandrinia Smith	Predator	11	0.46	13	0.88
Polistes sp.	Predator	08	0.91	11	0.75
Allorhynchium argentatum Fabricius	Predator	05	0.35	11	0.75
Family: Scoliidae					
Dielis sp.	Nectarivorous	01	0.06	00	0.00
Campsomeris collaris Guerin-Meneville	Parasitoids	03	0.19	00	0.00
Family: Megachilidae					
Megachile sp.	Florivorous	18	1.13	17	1.16
Family: Andrenidae					
Andrena sp.	Polyelectic	22	1.38	16	1.09
Family: Sphecidae					
Sceliphron madraspatanum Fabricius	Predator	03	0.19	06	0.41
Family: Ichneumonidae					
Coelicheumon sp.	Parasitoids	09	0.57	00	0.00
Family: Formicidae					
Camponotus sp.	Predator	32	2.01	18	1.22
ORDER- COLEOPTERA Family: Coccinellidae					
Coccinella septumpunctata (Linnaeus)	Predator	90	5.65	105	7.14
Oenopia sp.	Predator	26	1.63	18	1.22
Cheilomenes sexmaculata Fabricius	Predator	17	1.07	14	0.95
Family: Meloidae					
Mylabris cichorri Linnaeus	Predator	46	2.89	52	3.54
M. pustulata Thunberg	Predator	52	3.27	47	3.20

Cont...

Table 1. Diversity and relative abundance (%) of insect pollinators and trophic components during March, 2019 to February, 2021

Taxonomic composition	Trophic level	2019-2020		2020-2021	
		No. of individuals	Relative abundance (%)	No. of individuals	Relative abundance (%)
Family: Scarebaeidae					
Dung beetle sp.	Detritivores	07	0.44	05	0.34
Anomala sp.	Phytophagous	09	0.57	11	0.75
Phyllophaga sp.	Phytophagous	13	0.82	00	0.00
Family: Chrysomelidae					
Aulacophora sp.	Phytophagous	21	1.32	18	1.22
ORDER- DIPTERA Family: Muscidae					
Musca sp.	Saprophagous	22	1.38	27	1.84
Family: Syrphidae					
Eristalis sp.	Polyphagous	57	3.58	52	3.54
Episyrphus sp.	Polyphagous	62	3.89	75	5.10
Family: Asilidae					
Philodicus sp.	Predator	23	1.44	15	1.02
Family: Tachinidae					
Archytas sp.	Parasitoids	10	0.63	13	0.88
Family: Sarcophagidae					
Sarcophaga sp.	Saprophagous	17	1.07	00	0.00
ORDER- ORTHOPTERA Family: Acrididae					
Xenocatantops sp.	Phytophagous	23	1.44	19	1.29
Family: Tettigoniidae					
Elimaea sp.	Phytophagous	31	1.95	17	1.16
ORDER- HEMIPTERA Family: Pentatomidae					
Nezara viridula Linnaeus	Phytophagous	18	1.13	09	0.61
Family: Lygaedae					
Lygaeus sp.	Polyphagous	11	0.69	10	0.68
ORDER- THYSANOPTERA Family: Thripidae					
Thrips sp.	Predator	98	6.16	83	5.65
Total		1592	100	1470	100

emphasizing the dominance of phytophagous insects (Dev et al., 2009, Chouangthavy et al., 2017, Atencio et al., 2018, Ghani and Maalik 2020). The present study shows similar results, with phytophagous insects being dominant in species richness and abundance.

The Shannon-Wiener Diversity Index (H'), Evenness (E), and Margalef's Species Richness Index (d) were used to calculate the diversity of insect fauna collected (Tables 2 and 4). The maximum Species Diversity Index (H') was 3.981, Evenness (E) 0.9514, and Margalef's Richness Index (d) 11.33. Lepidopterans had the highest diversity index (H'=4.041), the highest species richness (d=8.719), and Thysanopterans had the highest evenness (E'=0.9966) (Table 4). Previous studies have reported a Shannon Diversity Index for entomofauna (H'=2.93 to 3.57) and Evenness (E'=0.721 to 0.947) in various crops in urban and croplands of Dera Ghazi Khan, Pakistan, indicating high diversity (Amber et al., 2015). Naz et al. (2020) reported a maximum Shannon Diversity Index (H'=2.195) and Evenness (E'=0.77768) of insect fauna from seven different agro-ecosystems in the Tarai region of Uttarakhand. Similar observations are documented for insect pollinators of

Table 2. Species diversity and species richness of insect fauna during March, 2019-2021

Months	2019-2020			2020-2021			2019-2021		
	Shannon Index (H')	Evenness (E')	Margalef (d)	Shannon Index (H')	Evenness (E')	Margalef (d)	Shannon Index (H')	Evenness (E')	Margalef (d)
March	2.945	0.7605	5.146	2.861	0.699	5.212	3.013	0.6562	5.636
April	3.511	0.8165	7.481	3.346	0.6922	7.481	3.52	0.7041	7.781
May	3.647	0.7994	8.556	3.64	0.7621	8.874	3.735	0.748	8.87
June	3.813	0.8231	9.914	3.859	0.8625	10.14	3.924	0.8036	10.19
July	3.78	0.8425	9.79	3.791	0.8522	9.977	3.924	0.7783	10.92
August	3.493	0.8218	8.146	3.572	0.8678	8.415	3.756	0.7637	10.07
September	3.913	0.7946	11.05	3.846	0.7933	10.47	3.981	0.7439	11.33
October	3.543	0.7515	8.911	3.288	0.705	7.784	3.592	0.6723	9.442
November	2.969	0.9273	5.539	2.345	0.8696	3.417	2.868	0.838	4.865
December	1.864	0.9211	2.415	1.735	0.9449	2.276	1.952	0.8805	2.422
January	1.56	0.9514	1.924	1.889	0.9448	2.731	2.119	0.925	2.824
February	1.72	0.9309	2.085	1.735	0.9449	2.276	1.923	0.8551	2.377

Table 3. Seasonal species diversity and richness of insect fauna in study area (Paharpani)

Season	2019-2020			2020-2021			2019-2021		
	Shannon Index (H')	Evenness (E')	Margalef (d)	Shannon Index (H')	Evenness (E')	Margalef (d)	Shannon Index (H')	Evenness (E')	Margalef (d)
March-June (Summer)	3.774	0.7024	9.169	3.686	0.6757	8.733	3.77	0.6477	8.979
July-October (Rainy)	3.934	0.7516	10.16	3.901	0.7611	9.876	3.987	0.709	10.37
November-February (Winter)	2.888	0.8549	4.757	2.546	0.797	3.796	2.905	0.7303	5.013

Table 4. Relative abundance, Species diversity, evenness and species richness of insect orders (Paharpani)

Orders	Relative abundance (%)	Shannon index (H')	Evenness (E')	Margalef (d)
Lepidoptera	35.76	4.041	0.9025	8.719
Hymenoptera	17.34	3.27	0.6924	5.793
Diptera	21.51	2.195	0.8167	1.689
Coleoptera	16.08	2.455	0.7281	2.446
Hemiptera	1.40	1.346	0.9606	0.775
Orthoptera	2.63	1.359	0.973	0.6667
Thysanoptera	5.28	0.6897	0.9966	0.1924
Total	100			

agricultural land (Duara and Kalita 2013, Das et al., 2018, Rekha et al., 2021, Arya et al., 2023).

CONCLUSION

The present study indicates that Lepidopteran and Hymenopteran insects are the major pollinators of agroecosystems. Other insect orders, such as Diptera, Coleoptera, and Thysanoptera, are also considered pollinators but are comparatively less important. Given the importance of these insects, it is necessary to conserve different species of pollinators. Although this was a preliminary attempt to report insect pollinators of Paharpani, Nainital, Uttarakhand, it will undoubtedly serve as baseline data for future researchers studying pollinators in the area.

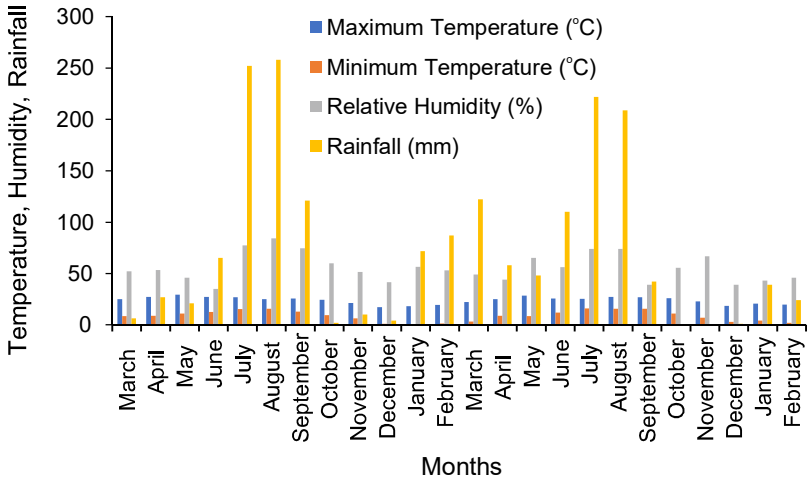

Fig. 5. Climatic data for study site March, 2019 to February, 2021

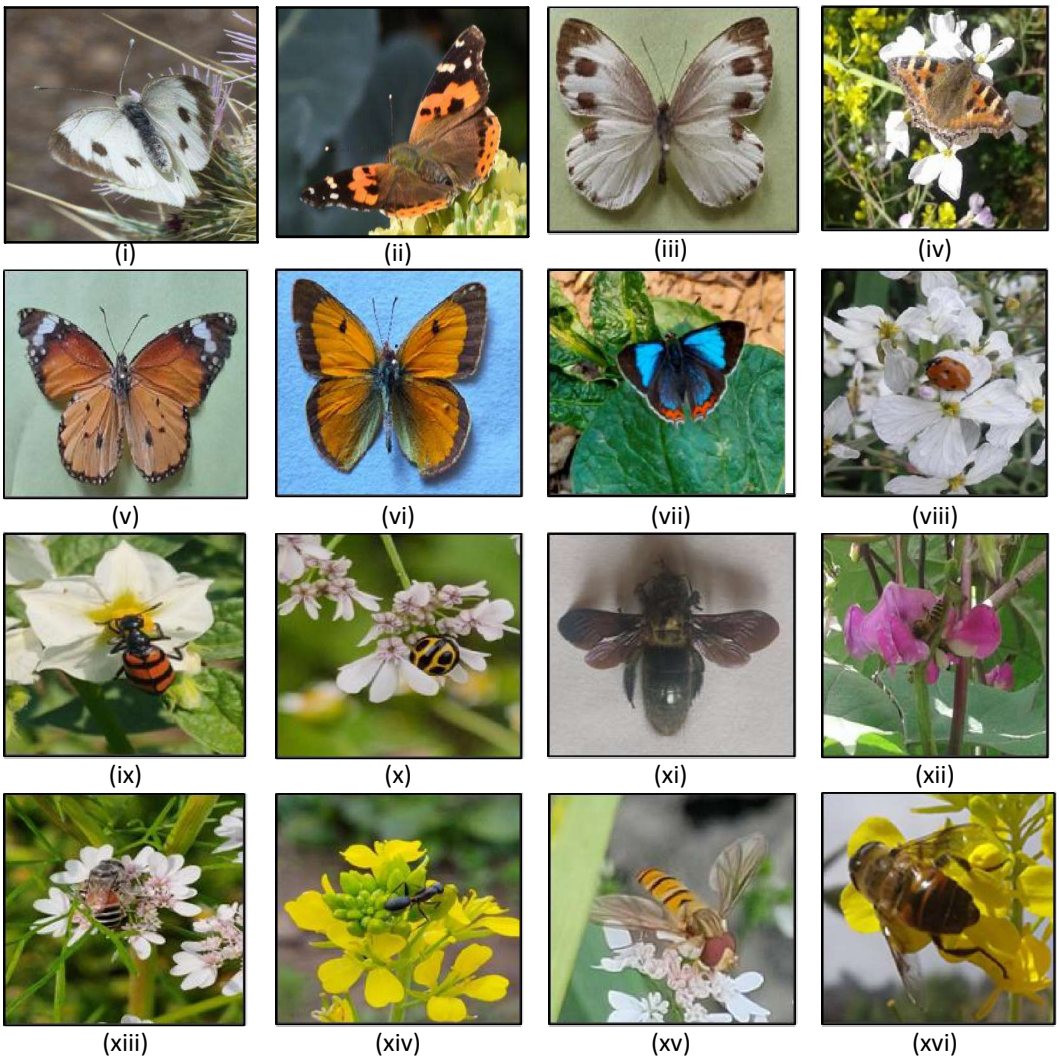

Plate 1. Insect pollinators: *(i) Pieris brassicae, (ii) Vanessa cardui, (iii) Pieris canidia indica, (iv) Aglais caschmirensis, (v) Danaus chrysippus, (vi) Colias fieldii, (vii) Heliophorus androcles, (viii) Coccinella septumpunctata (ix) Mylabris pustulata, (x) Oenopia sp., (xi) Xylocopa sp., (xii) Apis mellifera, (xiii) Apis florea, (xiv) Camponotus sp., (xv) Episyrphus sp., (xvi)Eristalis sp.*

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Antifeedant Activity of *Azadirachta indica* against *Spodoptera frugiperda* (J. E. Smith)

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***Abstract*:** In the dual choice test, feeding deterrence was recorded in the second instar larval stage of *Spodoptera frugiperda* when treated with the sub-lethal concentrations (LC_{30} and LC_{50}) of the neem-based formulations i.e., commercial neem formulation (0.15%), neem oil and neem seed kernel extract (NSKE).). The highest antifeedant activity was in NSKE (43.53 and 72.06 per cent) in comparison to the commercial neem formulation (27.37 and 58.97 per cent) and neem oil (17.51 and 50.59 per cent). The relative consumption index (RCI) was also high in case NSKE (0.808) followed by commercial neem formulations and neem oil. The higher antifeedant activity and RCI indicates high level of feeding deterrent.

Keywords: Antifeedant, Neem based formulations, Relative consumption index, *Spodoptera frugiperda*

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera; Noctuidae) is polyphagous, noxious insect-pest native to tropical and subtropical regions of the Americas. Although it is a key pest of maize crop, but also infest around 80 plant species including rice, sorghum, cotton, alfalfa, millet, peanut and other cultivated and wild plant species (Belay et al., 2012, Prasanna et al., 2018, Kammo et al., 2019, Sisay et al., 2019). There are two major strains of the fall armyworm i.e. the maize strain and rice strain (+6). Among these two, maize strain is more prevalent and feeds on maize stem and leaves (Bhusal and Chapagain 2020). Larval stage of this insect-pest is the voracious feeder which causes a huge damage to the host crop. This pest has extensive defoliation capacity making it one of the economically interesting pest (Barbosa et al., 2018).)

Fall armyworm is a migrating pest which causes significantly high damage in host crop and can fly up to 1600 Km in time period of 30 hours (Bhusal and Chapagain 2020). It has migrated to continents other than Americas, being detected first in Central and Western Africa (Benin, Nigeria, Sao Tome and Principe, and Togo) in 2016 and reported further in Southern Africa (except Lesotho), Madagascar and Seychelles (Goergen et al., 2016, FAO 2018, Bhusal and Chapagain 2020). In 2018, fall army worm was reported in almost all Sub-Saharan African countries, except Djibouti, Eritrea and Lesotho (FAO 2018). It is distributed in 40 sub- Saharan African countries. The first incidence of FAW was reported in July 2018 in India from Karnataka (Saranbassappa et al., 2018) and thereafter reported from states Bihar, Chhattisgarh, Gujarat, Maharashtra, Andhra Pradesh, Odisha, Tamil Nadu and West Bengal (Deole and Paul 2018, CABI 2019, Bhusal and Chapagain 2020).The irrational use of synthetic insecticides to control insect pests has often resulted in problems like chemical residues in food, biological disequilibrium, intoxication to non target organisms, development of insect resistance (Gutierrez-Moreno et al., 2019) The search for alternative methods includes the use of natural products that are both effective and less environmentally aggressive, such as plant extracts (Roel and Vendramim 2006).

Azadirachta indica A. Juss. (Meliaceae), commonly known as neem or Indian lilac, is indigenous plant of the Indian subcontinent and Southeast Asia which is known for thousand years for antiseptical properties (Roel et al., 2010, Campos et al., 2016, Duarte et al., 2019). Every plant part i.e. root bark, fruits, twigs, seeds, flowers and stem produces more than 100 active biological compounds, mainly, terpenoids (Copping and Duke 2007, Shannag et al., 2015, Campos et al., 2016) which have repellent, insecticidal and antifeedant properties for control of many insect-pests (Kubo and Klocke 1982, Kumar and Poehling 2007, Shannag et al., 2015, Duarte et al., 2019). Azadirachtin, a steroid-like tetranortriterpenoid is the primary active compound responsible for wide range of bioactivity on many insect-pests (Mordue (Luntz) 2004, Mordue (Luntz) 2005, França et al., 2017). Azadirachtin also affects both juvenile and ecdysteroid hormone levels by inhibiting release of morphogenetic peptides from the brain which results in a blockage of ecdysteroids synthesis and a regulation of the release of juvenile hormone in insects (Mordue (Luntz) and Nisbet 2000, Garcia et al., 2006, Abdullah and Subramanian 2008, Morgan 2009). The present study was conducted to determine the antifeedant activity of *A. indica* formulations against *S. frugiperda.*

MATERIAL AND METHODS

The *S. frugiperda* population was collected in perforated polythene bags from maize fields of Punjab Agricultural University, Ludhiana, Punjab during 2020-21 and brought to the laboratory. The culture was maintained on the maize leaves in incubator at the temperature and relative humidity of 26±1oC and 65±5 per cent respectively. The larvae were kept in small plastic vials (2 x 2.5 cm). Leaves were changed daily. The larvae developed to the pupal stage and then the pupae were separated in battery jars. The sand layer in jar up to 5 cm for pupation after six instar larvae. The separate glass jars covered with muslin cloth were used to transfer the collected pupae and these jars secured with rubber bands till the adult emergence stage. The emerging adult were sexed and transferred

Table 1. Antifeedant effect and relative consumption index of neem based formulation on the *S. frugiperda*

Formulation	Concentration (%)	Antifeedant activity (%)	Relative consumption index (RCI)
Commercial neem (0.15%)	LC_{30} (0.000169)	27.37	0.173
	LC_{50} (0.000288)	58.97	0.605
Neem oil	LC_{30} (0.045)	17.51	0.084
	LC_{50} (0.238)	50.59	0.371
NSKE	LC_{30} (0.695)	43.53	0.449
	LC_{50} (1.348)	72.06	0.808

into hollow glass cylinders which were placed on top of pots containing maize plants. Cotton swab dipped in a 10 per cent solution of honey was hung from the top of the muslin cloth covering the mouth of the glass cylinder. The adults were sexed based on differences in the size and shape of the wings of male and female moths. The female moths are slightly smaller than males and the forewings have fawn-colored spots towards the center and white patches at the apical margin in adult male moths, but without spot and brown forewings in adult female moths. Adults, after mating, lay eggs on the maize leaves. The oval/round and dirty white eggs were laid in clusters and covered with hairs. To facilitate regular oviposition the maize leaves with eggs was removed on daily basis and replaced with the fresh one. All laboratory glassware used in the experiments was carefully cleaned using detergent, disinfected with 2% formalin, and subsequently dried in an oven at 30°C for 8 hours to prevent any microbial contamination in the insect cultures. The neem-based treatments were commercial neem formulation (Neem Kavach), neem oil, and 5% neem seed kernel extract (NSKE).

Determination of antifeedant effects of *A. indica* on *S. frugiperda:* The feeding performance of test larvae was evaluated by employing a 'dual choice' test. Maize leaf portion was cut into two halves. One portion was treated by dipping in test formulations (LC_{30} and LC_{50}) and the second portion was dipped in distilled water which was served as control. After drying on paper towels both portions were placed in Petri plate (9 cm diameter). Ten test larvae were released in the center of the plate and feeding exposure of 24 hours was given. There were five replications for each treatment. Per cent antifeedant activity and relative consumption index (RCI) was calculated by using the following formula is given by Saleh et al. (1986) and Sandhu and Singh (2000), respectively.

$$\text{Antifeedent activity (\%)} = \left(1 - \frac{\text{(Per cent treated area consumed)}}{\text{(Per cent control area consumed)}} \times 100\right)$$

$$RCI = \frac{C - T}{C + T}$$

Where, C and T refer to percent leaf eaten in control and treated leaf portions.

Statistical analysis: The data were analyzed using the statistical software package CPCS1 and SPSS 16.0 statistical software.

RESULTS AND DISCUSSION

In the dual choice test, feeding deterrence on the second instar larvae of *S. frugiperda* was observed with the sub-lethal concentrations (LC_{30} and LC_{50}) of the neem based formulations, i.e., commercial neem formulation (0.15%), neem oil and NSKE were observed for antifeedant effects. The maximum antifeedant activity was reported at the concentration LC_{50} of the NSKE (72.06 per cent) as a comparison to the other LC_{50} concentrations of commercial neem formulation (0.15%) and neem oil, i.e. 58.97 and 50.59 per cent, respectively on larvae of the *S. frugiperda*. Similarly, the highest antifeedant effect wan in LC_{30} concentration NSKE (43.53 per cent) followed by commercial neem formulations (0.15%) and neem oil. However, the LC_{30} and LC_{50} values of NSKE showed a more pronounced effect on *S. frugiperda* larvae compared to the commercial neem formulation (0.15%) and neem oil derived from *A. indica*. The higher antifeedant activity indicates the higher feeding deterrence. The sub-lethal concentration LC50 significantly increased the antifeedant activity in comparison to LC30 in three different neem based formulations (Table 1). Three relative consumption indices (RCIs) were significantly highest in NSKE (0.808), followed by commercial neem formulations (0.15%) and neem oil). Similarly, the leaves treated with the LC30 concentration had the highest relative consumption index of NSKE as compared to other LC30 concentrations of commercial neem formulations (0.15%) and neem oil (Table 1). The sub-lethal concentration LC50 significantly increased the relative consumption index in comparison to LC30 in three different neem based formulations.

Ghoneim and Hamadah (2017) also observed highest antifeedant activity of Nimbecidine (0.03% Azadirachtin) against the second instar larval stage of *Spodoptera littoralis*. Wondafrash et al., (2012) concluded that neem seed extract had higher antifeedant action than neem leaf extract, against *Helicoverpa armigera* larvae.. In *Spodoptera eridania* (second instar), short-term feeding (2 days) on food treated with Azatrol, a commercial neem-based azadirachtin formulation, resulted in a reduced relative consumption rate (Shannag et al., 2015). Prasoona et al. (2022) reported strong antifeedant activity of neem seed kernel extract against third instar larvae of *S. frugiperda*.

CONCLUSION

Neem seed kernel extract demonstrated the most potent antifeedant effect among all neem-based formulations, although each formulation exhibited some degree of antifeedant activity. The proven safety of these neem formulations for non-target organisms and mammals, along with their deterrent effects on *S. frugiperda*, makes them promising candidates for field trials in the pest's management.

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Breynia retusa (Phyllanthaceae): New Larval Host Plant for *Eurema andersonii* (Moore 1886) (Lepidoptera: Pieridae) from India

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Abstract: The life cycle of Eurema andersonii from the oviposition to the emergence of adult completed on 27-28 days. During the present study Eurema andersonii completed its complete life cycle on Breynia retusa. therefore, Breynia retusa is reported here as a new larval host plant of Eurema andersonii from Arunachal Pradesh India.

Keywords: *Eurema andersonii, Breynia retusa,* Phyllanthaceae, Vijaynagar, Arunachal Pradesh

Eurema andersonii (Moore 1886) belongs to the Pieridae family and is commonly known as the One-spot Grass Yellow and is distributed in India (Southern India to Southern Maharastra, Uttarakhand, Sikkim-Arunachal Pradesh, NE, Andaman Island), Bhutan, Myanmar, Sri Lanka (Kehimkar 2016) and Nepal (Van der Poel 2020). The genus *Eurema* Hübner, 1819 consists of approximately 70 species with different geographical distributions but to date, India has reported 7 species *viz., E. andersonii, E. blanda, E. brigitta, E. hecabe, E. laeta, E. nilgiriensis* and *E. simulatrix*. The species *E. andersonii* comprises three subspecies: *evansi, jordani and shimai.* The subspecies *E. a. jordani* Corbet & Pendlebury, 1932 is distributed from Uttarakhand to N.E. India, and West Bengal (Varshney & Smetacek 2015). Initially, *E. jordani* was recognised as a separate species, Jordan's Grass Yellow (Talbot 1939).

The previously reported larval host plants were *Ventilago goughii* (Yata and Gaonkar 1999, Nitin et al 2018) and *Ventilago maderaspatana* (Naik and Mustak 2020) which belongs to the family Rhamnaceae. However, *Breynia retusa* (Dennst.) Alston, which belongs to the family Phyllanthaceae, has not been previously reported elsewhere from India. The distribution and abundance of insects are significantly influenced by the availability and abundance of their host plants (Knops et al 1999). Therefore, understanding the intricate relationships between butterflies and their host plants will help in the development of targeted conservation strategies that nurture and protect these delicate miniature fluttering jewels.

MATERIAL AND METHODS

The survey was conducted over 2 years, from January 2021 to December 2022, in the remotest Eastern circle of Vijaynagar, Arunachal Pradesh, India (Fig. 1). The survey was carried out in different habitats such as agricultural fields, roadside vegetation, human habitat areas, forest patches, vegetation near the riverbed and stream and inside the forest area where sun rays could pass. Observations of oviposition behaviour were conducted, where an adult female butterfly was observed depositing eggs under the surface of young and mature leaves of *Breynia retusa*. Eggs and host plants were subsequently collected for rearing in the laboratory to facilitate the examination of the entire life cycle. The photographic records were taken at each developmental stage, and documentation was done by a Canon EOS 80D camera equipped with a Tamron 90 mm macro lens. The emergence of an adult butterfly was identified (Kehimkar 2016).

RESULTS AND DISCUSSION

The three adult females of *Eurema andersonii* were observed depositing eggs singly under the surface of young leaves and shoots of *Breynia retusa* at village Mazgaon (27°13'37.59"N; 96°58'32.51"E) of Vijaynagar, Arunachal Pradesh on November 08, 2021. A few eggs were also observed under the surface of the mature leaves. The six eggs were collected and reared under laboratory conditions to document their life stages. The remaining eggs were reared on the larval host plant in the natural habitat. The collected eggs and eggs in the larval host plant hatched after 4-5 days. Newly hatched caterpillars started feeding on their eggshells as their first meal, later they began to feed on the tender leaf tissue except the veins. After moulting to third instars, all the caterpillars including the caterpillars that were left on their natural habitat died, as *Breynia retusa* is very tender and due to the extreme cold in the region caused all the leaves to shed from the host plants.

To confirm the new larval host plant for *Eurema*

andersonii, a repeat rearing of the life cycle was performed to ensure the accuracy of host plant identification and facilitate a more comprehensive understanding of the life history of *Eurema andersonii*. A subsequent observation on 24 August 2022, at Buddhamandir (27°12'36.02" N and 96°59'38.13" E), revealed a single adult *E. andersonii* engaged in oviposition on the upper surface of the young leaves of *Breynia retusa*. Three freshly laid eggs were collected to monitor different stages under laboratory conditions. Hatching of larvae occurred 4-5 days after oviposition. On 29 August 2022, all three collected eggs were successfully hatched and commenced feeding on their eggshells as a first nutrient source before transitioning to leaf tissue consumption. On 8 September 2022, the larvae ceased feeding and searched for suitable pupation sites. On 9 September 2022, the larvae decreased in length and subsequently secured themselves to a substrate via silk-like threads before entering the pupal stage. On 19 September 2022, the pupae became translucent, indicating imminent adult emergence. On 20 September, 2022, an adult *Eurema andersonii* emerged at 08:00 hours, dried its wings and achieved flight readiness by 10:30 hours. The life cycle from the oviposition to the emergence of adult took 27 to 28 days. Photographic documentation of various life stages conducted throughout the rearing process (Plate 1). The comprehensive review of the literature revealed that the Phyllanthaceae family had not been previously documented as a larval host plant for *Eurema andersonii*. Therefore, this study report *Breynia retusa* (Phyllanthaceae) as a new larval host plant for *Eurema andersonii* from Vijaynagar, Arunachal Pradesh, India (Plate 2).

Breynia retusa (Dennst.) Alston is a compact shrub, reaching heights of 1-2 m, with spreading branches and elliptical leaves measuring 1.25-2.5 cm in length. The small

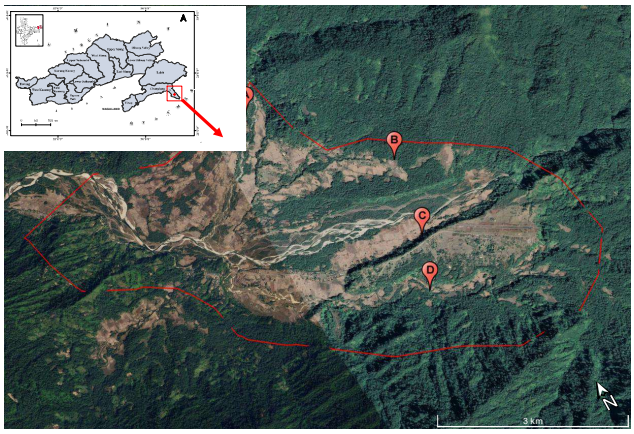

Fig. 1. Maps of Vijaynagar (District Changlang) Arunachal Pradesh: A-Mazgaon; B-Buddhamandir; C-Daragaon; D-Daowdi

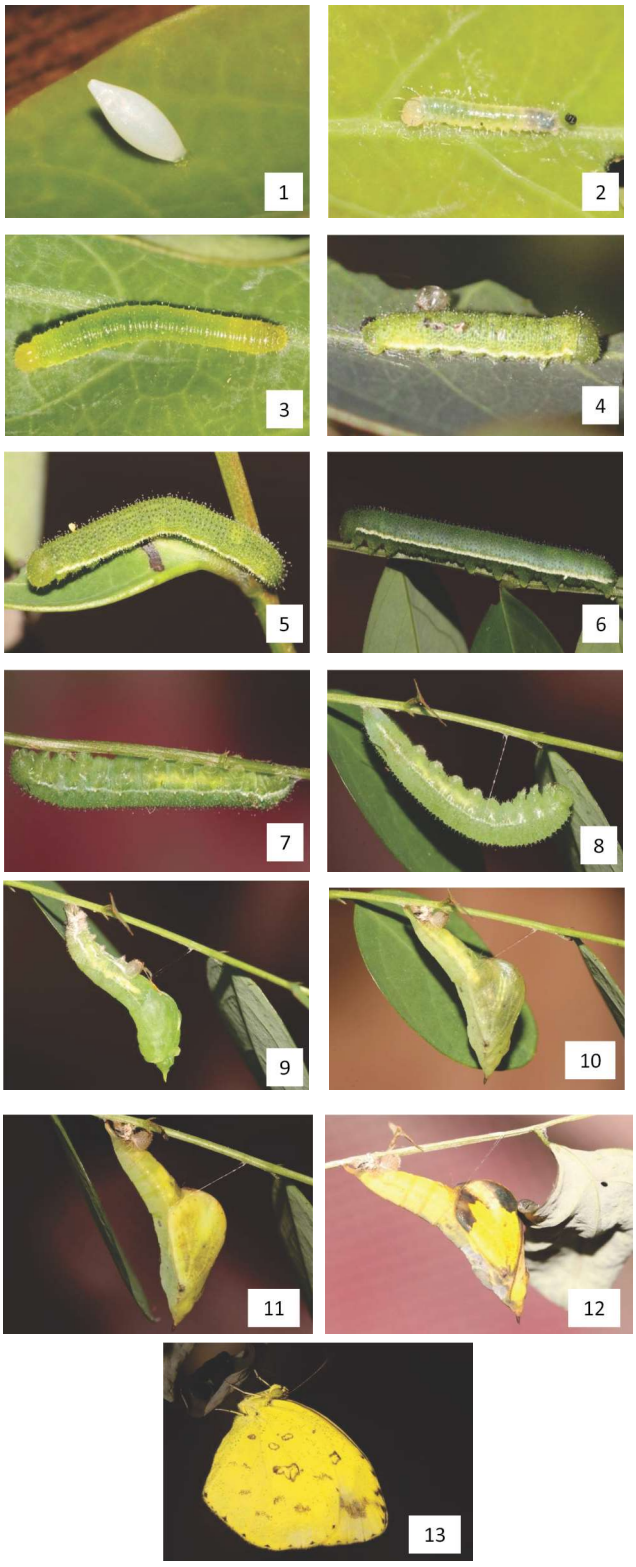

Plate 1. 1- Egg; 2-1st instar caterpillar; 3- 2nd instar caterpillar; 4- 3rd instar caterpillar; 5- 4th instar caterpillar; 6- 5th instar caterpillar; 7- prepupatory caterpillar; 8- prepupatory caterpillar spins a silk pad and silk girdle; 9-Pupation; 10- Pupa; 11- Pupa become translucent for enclosed; 12- Mature pupa; 13- Newly enclosed *Eurema andersonii* (Moore 1886)

Table 1. Details of the sampling sites of the host plant in Vijaynagar, Arunachal Pradesh

Site	Village	Latitude (N)	Longitude (E)	Altitude (m)
A	Mazgaon	27°13'37.59"N	96°58'32.51"E	1242 m
B	Buddhamandir	27°12'36.02"N	96°59'38.13"E	1296 m
C	Daragaon	27°11'51.80"N	96°59'29.99"E	1216 m
D	Daowdi	27°11'23.41"N	96°59'17.68"E	1220 m

Plate 2. 1, 2 -Plant of *Breynia retusa* (Phyllanthaceae); 3-Flowers of *Breynia retusa*

flowers are axillary and borne on thin pedicels, with males in the lower axils and females in the upper axils and the male flowers are pale yellowish. The fruit is depressed-globose and approximately 13-17 mm in diameter (Bhagyasri et al 2017). It is distributed in India, Bhutan, China, Malaysia, Cambodia, Laos, Vietnam, Thailand, Nepal (Kumar and Balakrishnan 1996) and Bangladesh (Bhagyasri et al 2017). In the present study, *Breynia retusa* was predominantly documented near water sources, thriving within areas of fragmented shrub vegetation (Table 1).

AUTHOR'S CONTRIBUTION

RL analysed the data and performed the experiments. RG contributed to larval host plant identification. RU conducted species identification and verification. RA provided overall guidance and supervision. All the authors reviewed, edited, and approved the final manuscript.

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Ultrastructure of Larval Instars of *Artaxa vitellina* Kollar and *Maeoproctis latifascia* Walker (Insecta: Lepidoptera: Lymantriinae) by using Scanning Electron Microscopy from Himachal, India

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***Abstract*:** The current study delves into comparing the ultrastructure of antennae and mouthparts of two moth larvae, *Artaxa vitellina* Kollar and *Maeoproctis latifascia* Walker, from the same subfamily, Lymantriinae (Family: Erebidae). It identifies distinct differences in their sensory structures, particularly in the distribution of medial sensilla of the maxillary palp and sensilla basiconica of the antenna across larval stages. Moreover, it underscores the significance of incorporating larval morphological characteristics to enhance taxonomic classification and offer insights for future electrophysiological studies on pest behaviour and phylogenetic analyses.

Keywords: Lepidoptera**,** Moths, Larvae, Caterpillars, Ultrastructure, Sensilla, Scanning electron microscopy, Statistical analysis, Taxonomy, Immature stages

Lepidoptera is the third-largest group of insects, mainly consisting of moths. These insects undergo complete metamorphosis, progressing through egg, larva, pupa, and adult stages. Larvae are particularly useful for taxonomic classification. This study focuses on using the distribution of sensilla (sensory organs) on larvae's antennae and mouthparts as a basis for classification. Sensilla are found on various body parts, but antennae and mouthparts are key sensory appendages responding to a range of stimuli. The family Erebidae is one of the most diverse families within the order Lepidoptera, comprising over 25,000 described species distributed worldwide (Zahiri et al., 2011) and includes a wide array of moths, ranging from large, robust species to smaller, cryptic forms. The current literature indicates a focus on studying the ultrastructure of only the final instars of larval stages, overlooking the initial stages. This study aims to utilize larval characteristics from all stages for identification purposes.

MATERIAL AND METHODS

Collection and identification: The eggs and different instars were collected from different localities of district Kullu, Himachal Pradesh, India (31°57′36″N, 77°6′0″E, 1279m) and sample collection of lab was utilised for preparation for scanning electron microscopy.

Scanning Electron Microscopy (SEM): The head of each larval instar was separated and placed in Carnoy's fixative solution (95% ethanol and glacial acetic acid with ratio of 3:1) for 3 hours in case of first 3 instars and for 12 hours for later instars and then kept in 2% glutaraldehyde at 4°C for 2 hours in case of initial three instars and overnight for later instars. The following day, the samples were cleaned twice with a phosphate buffer (each for 10 min) and washed in 100% ethanol solution (three times, each washing for 2 min). The antennae and mouthparts were then dehydrated through a graded ethanol series of 30, 50, 70, 80, and 90% (for 15 min each) and then fully dehydrated in 100% ethanol (three times for 30 min each), and lastly cleaned in isoamyl acetate (three times for 10 min each). Samples were air dried for 1 hour before performing Scanning Electron microscopy. Different aged instars were preserved in glass vials containing 70% alcohol and glycerol in the ratio of 8:2. Samples were examined and photographed for their ultrastructure studies under scanning electron microscope (JEOL) JSM-6100 in the Instrumentation Centre, Punjabi university, Patiala.

Analysis of SEM micrographs: The sensilla were identified from SEM micrographs based on terminologies proposed by Schneider (1964), Zacharuk (1980, 1991) and Grimes (1986 a, 1986b). Images were labelled using Adobe Photoshop CS4 software. Metric analyses were performed using ImageJ software (Li et al*.,* 2018).

RESULTS AND DISCUSSION

***Artaxa vitellina* Kollar (Figs. 1-5):** Five instars were observed in a single generation. Both the second and final segments of the antenna displayed the expected sensilla. The second segment featured a pair of sensilla chaetica (C1-C2) and three sensilla basiconica (B1-B3), while the final segment had three sensilla basiconica (B4-B6) along with a sensilla styloconica (Sty). However, sensilla B5 and B6 on

the final segment of the antenna were positioned together and not alternately arranged on either side of the sensilla styloconica. The mandibles consistently exhibited a pair of six sensilla chaetica (C1-C12) in all larval instars. Sensilla on the galea were complete. In the case of the maxillary palp, all sensilla basiconica (A1-A3, L1-L2, and M1-M2) were observed in all larval instars except for the medial sensilla basiconica (M1-M2), which were absent in the first instar. The labial palp maintained a pair of sensilla chaetica (C) and sensilla styloconica (Sty) along with a spindle-shaped spinneret across all instars.

***Maeoproctis latifascia* Walker (Figs. 6-10):** Five instars were observed within a single generation. Across all larval instars, the second segment of the antenna consistently exhibited a pair of sensilla chaetica (C1-C2). Furthermore, three sensilla basiconia (B1-B3) were consistently present on the antenna's second segment in all stages, except for sensillum B3, which was not observed in the third and fourth instar, possibly due to its concealed location. In the final segment of the antenna, sensillum B5 was not observed in first, third and fifth instars. Similarly, sensilla B6 was observed only in first two instars. Positions of sensilla B5 and B6 probably make it hard to observe them as large sensilla basiconica B4 is positioned just in front of them. Sensilla B5 and B6 were positioned close together in proximity, as observed in second instar. The mandibles consistently displayed a pair of six sensilla chaetica (C1-C12) in all larval instars. On the galea, three sensilla trichodea (ST1-ST3) and two sensilla styloconica (LSS-MSS) were observed. Similarly, the maxillary palp exhibited eight sensilla basiconica, including three apical (A1-A3), two lateral (L1-L2), and two medial (M1-M2) sensilla in all larval instars, along with sensilla campaniformia (SC) and sensilla digitiformia (SD), which were observed in only two instars due to their visibility being angle-dependent. The labial palp consistently displayed a pair of sensilla chaetica (C) and sensilla styloconica (Sty) in addition to a spindle-shaped spinneret across all larval instars.

The present study provides a comparative account of the ultrastructural characteristics of the antennae and mouthparts of the larval instars of *Artaxa vitellina* and *Maeoproctis latifascia* using scanning electron microscopy. The observations in this study largely align with previous research on lepidopteran larvae, particularly concerning the general organization and presence of key sensilla types. The structural components of the antennae and mouthparts, including the presence of sensilla basiconica, sensilla chaetica, and sensilla styloconica, correspond with findings reported in other studies on lepidopteran larvae (Lin 1997, Fanger and Naumann 2001, Shields 2009, Song et al., 2014). However, species-specific differences in sensilla distribution and morphology were evident, emphasizing the importance of ultrastructural characteristics in taxonomic identification and ecological adaptation. The presence of sensilla campaniformia and sensilla digitiformia across all larval instars of *Artaxa vitellina*, compared to their occurrence in only select instars of *Maeoproctis latifascia*, suggests variations in mechanosensory adaptation. Previous studies have demonstrated the importance of such sensilla in detecting mechanical stimuli, particularly in larvae that engage in exploratory behaviours (Shields 2009). The consistent presence of these sensilla in *Artaxa vitellina* may

Table 1. Comparative account of observations made in all larval stages of the species studied:

Sensilla type	*Artaxa vitellina*	*Maeoproctis latifascia*
Sensilla campaniformia and sensilla digitiformia	Present in all instars	Observed only in two instars
Sensilla M1 and M2 of maxillary palp	Absent in first instar	Present in all instars
Sensilla B5 and B6	Observed in all instars	Observed in few instars
Shape of spinneret	Spinneret becomes elongated with a pointed tip	Spinneret remains broad till last instar

Table 2. Metrical analysis of sensilla of antennae of different aged instars of *Artaxa vitellina* (Mean length±SD micrometers)

Sensilla type	1st instar	2nd instar	3rd instar	4th instar	5th instar
B1	15.81±0.64	23.76±0.21	29.55±0.59	38.62±0.22	33.40±0.35
B2	8.51±0.93	9.73±0.66	15.42±2.25	22.58±0.43	22.76±0.79
B3	1.89±0.16	1.89±0.33	8.11±0.18	10.88±0.83	9.20±0.68
B4	12.40±0.32	14.16± 1.05	19.93±0.10	24.60±0.13	33.45±0.29
B5	3.08±0.34	4.38±0.32	4.25±0.19	2.27±0.34	8.40±0.48
B6	7.23±0.24	7.18±0.21	8.53±0.34	6.31±0.16	11.25±0.37

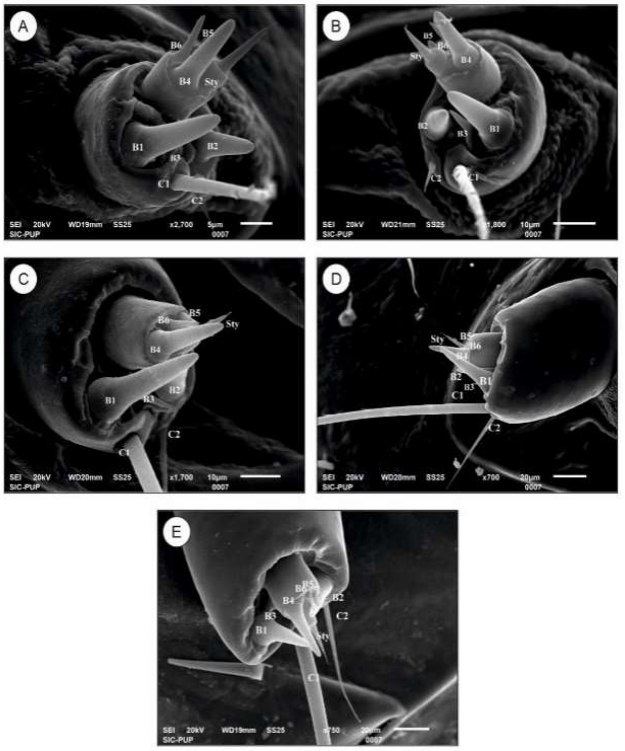

Fig. 1. Morphology and structure of sensilla on antenna in all larval instars of *A. vitellina.* (A) to (E) antenna of first to fifth instar respectively

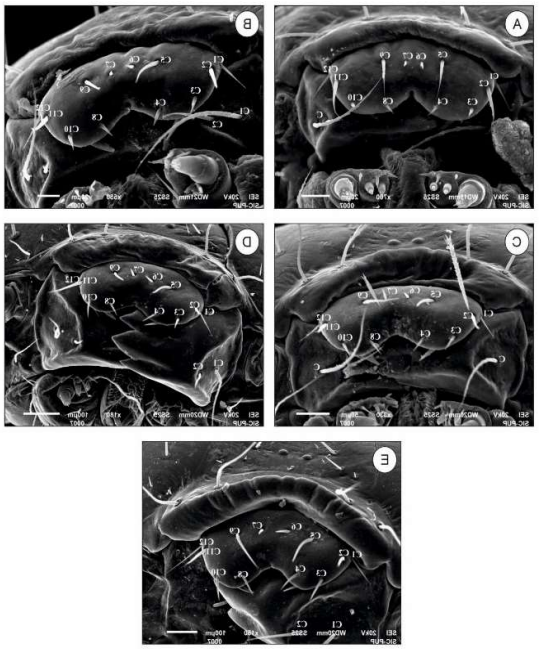

Fig. 2. Morphology and structure of sensilla on labrum and mandibles in all larval instars of *A.vitellina.* (A) to (E) labrum and mandibles of first to fifth instar respectively

indicate a more active role in environmental sensing across developmental stages.

Further, the absence of sensilla M1 and M2 in the first instar of *Artaxa vitellina* but their presence in all instars of

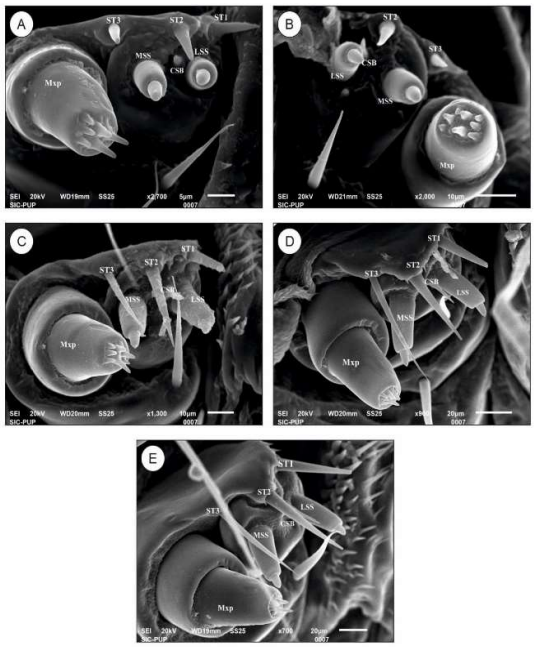

Fig. 3. Morphology and structure of sensilla on galea in all larval instars of *A. vitellina*. (A) to (E) galea of first to fifth instar respectively

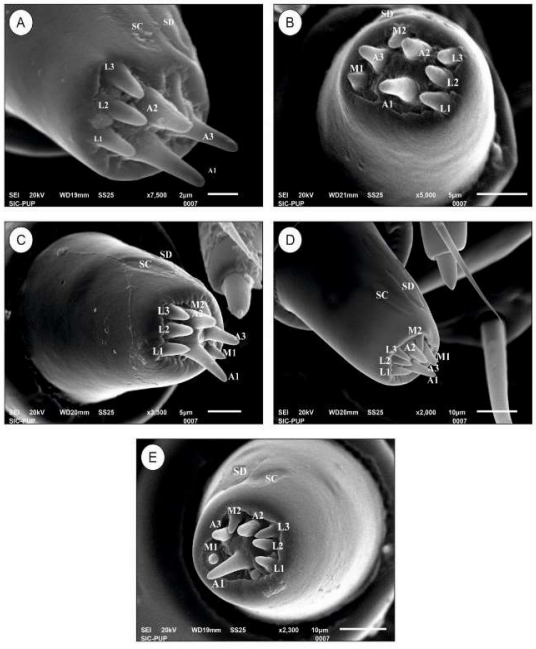

Fig. 4. Morphology and structure of sensilla on maxillary palp in all larval instars of *A. vitellina*. (A) to (E) maxillary palp of first to fifth instar respectively

Maeoproctis latifascia highlights interspecific differences in sensory organ development. Similar observations have been documented in other Lepidoptera, where the ontogeny of sensilla varies among species due to differences in feeding strategies and habitat preferences (Grimes and Neunzig 1986). The arrangement of sensilla basiconica on the antennae of both species also aligns with previous findings in related taxa. The alternate arrangement of sensilla B5 and

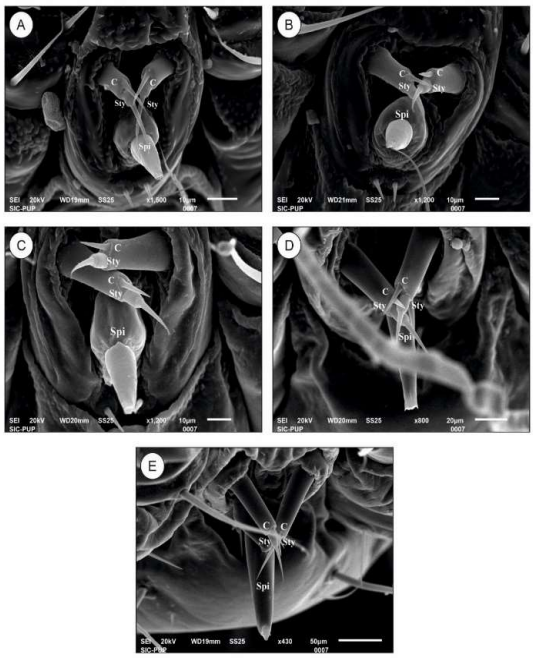

Fig. 5. Morphology and structure of sensilla on labial palp in all larval instars of *A.vitellina*. to (E) labial palp of first to fifth instar respectively

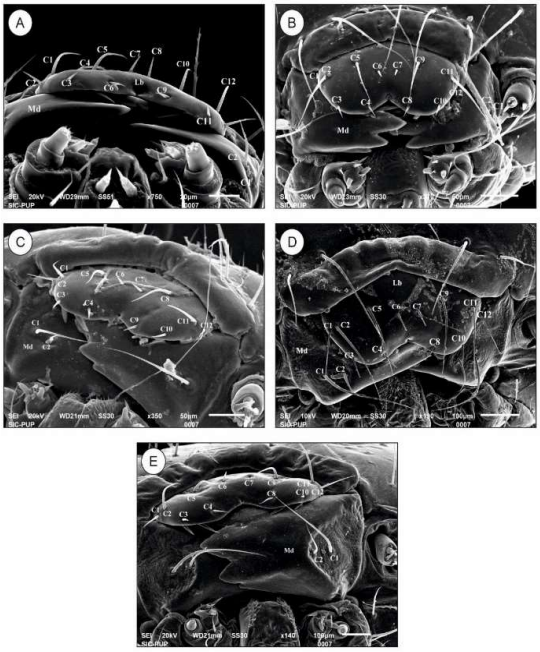

Fig. 7. Morphology and structure of sensilla on labrum and mandibles in all larval instars of *M. latifascia* (A) to (E) labrum and mandibles of first to fifth instar respectively

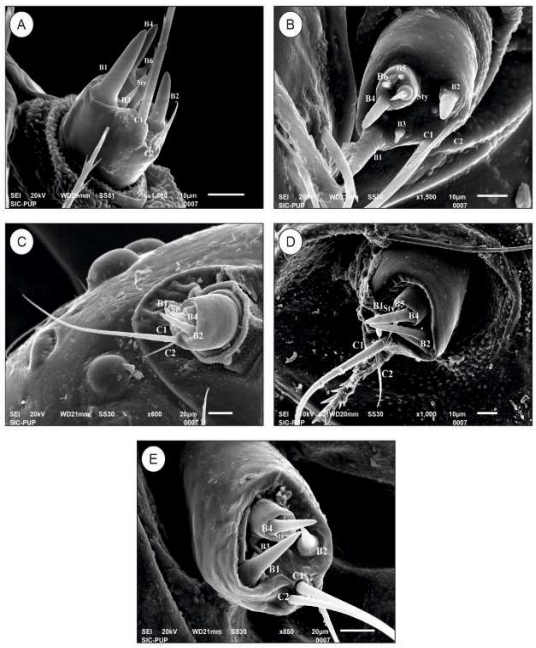

Fig. 6. Morphology and structure of sensilla on antenna in all larval instars of *M. latifascia*. (A) to (E) antenna of first to fifth instar respectively

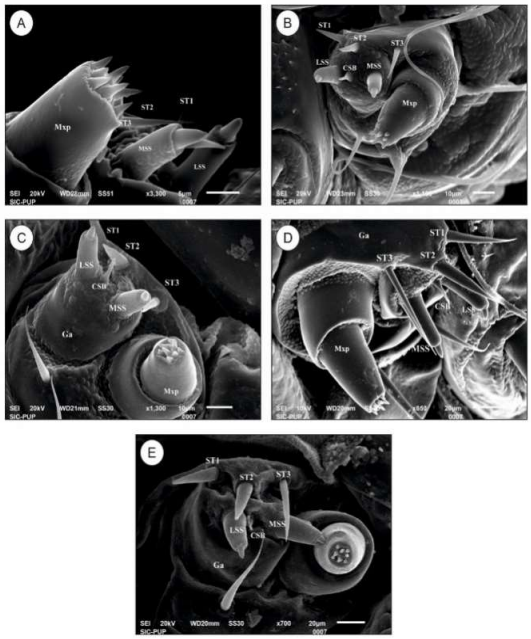

Fig. 8. Morphology and structure of sensilla on galea in all larval instars of *M. latifascia*. (A) to (E) galea of first to fifth instar respectively

B6 observed in some instars of *Artaxa vitellina* differs from *Maeoproctis latifascia*, where sensilla B5 was not observed in certain instars. This disparity suggests functional modifications in the sensory system, potentially linked to species-specific ecological interactions (Schneider 1964, Zacharuk 1991). Another noteworthy observation is the elongation of the spinneret with a pointed tip in *Artaxa vitellina*, whereas in *Maeoproctis latifascia*, the spinneret remains broad until the final instar. Similar morphological differences in spinneret structure have been reported in other

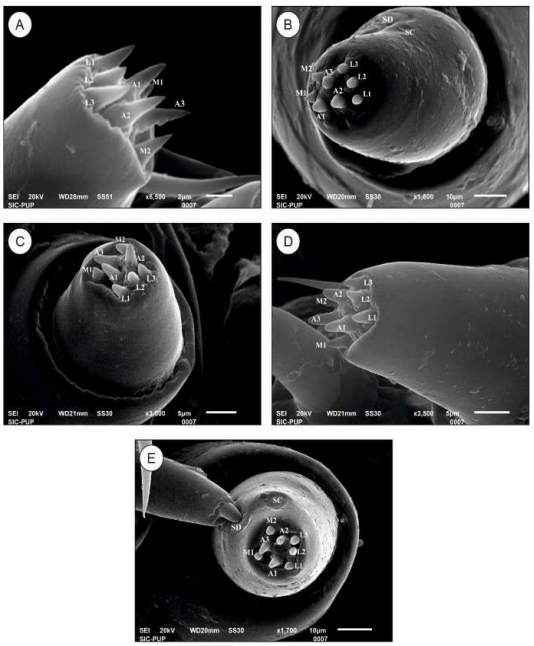

Fig. 9. Morphology and structure of sensilla on maxillary palp in all larval instars of *M. latifascia*. (A)to (E) maxillary palp of first to fifth instar respectively

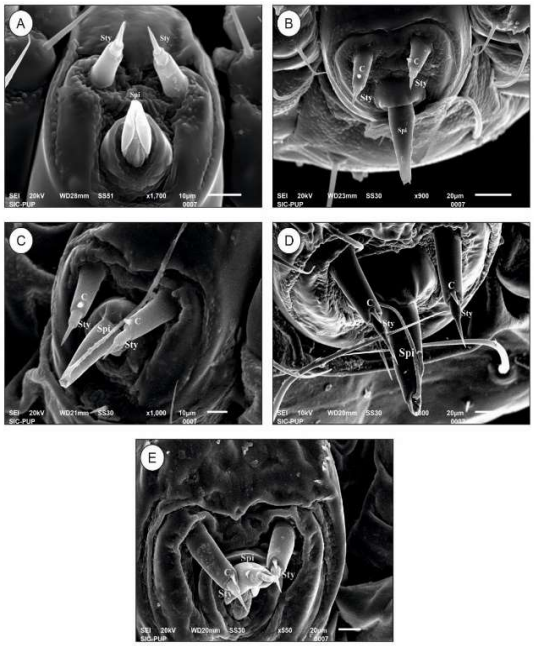

Fig. 10. Morphology and structure of sensilla on labial palp in all larval instars of *M. latifascia*. (A) to (E) labial palp of first to fifth instar respectively

Table 3. Metrical analysis of mouthparts and its sensilla of different aged instars of *A. vitellina* (Mean length±SD micrometers)

Name of structures	1st instar	2nd instar	3rd instar	4th instar	5th instar
Distal segment of Mxp	11.87±0.58	8.71±1.42	21.37±0.84	38.04±0.35	53.82±0.44
Galea					
ST1	8.67±0.16	16.74±1.60	14.38±0.84	30.64±0.58	54.28± 1.04
ST2	6.35±0.01	6.30±0.21	29.91± 0.15	42.28±0.39	65.54±1.07
ST3	2.98±0.48	4.47±0.03	24.83±0.57	62.85±0.46	73.60±1.38
Sensilla of maxillary palp					
A1	5.26±0.17	3.67±0.13	7.82±0.62	8.00± 0.45	9.81±0.41
A2	3.76±0.04	2.22±0.08	4.76±0.18	7.28±0.06	4.55±0.56
A3	4.84±0.15	2.94±0.034	5.44±0.05	6.94±0.41	3.27±0.29
L1	2.64±0.05	3.03±0.02	4.64±0.06	4.89±0.11	4.66±0.14
L2	2.64±0.02	4.15±0.25	3.32±0.10	5.46±0.18	4.08±0.29
L3	2.74±0.17	3.82±0.08	3.17±0.25	4.44±0.09	4.47±0.15
M1	Not visible	1.62±0.03	3.40±0.07	4.21±0.45	1.95±0.10
M2	Not visible	2.15±0.02	2.54±0.19	4.27±0.18	4.47±0.04
Sensilla of labial palp					
C	12.94±1.50	7.86±2.71	12.55±1.25	17.16±5.77	26.91±1.61
Sty	20.74±0.98	13.82±1.60	23.92±0.33	33.16±2.34	56.53±2.354

Table 4. Metrical analysis of sensilla of antennae of different aged instars of *M,latifascia* (Mean length±SD micrometers)

Sensilla type	1st instar	2nd instar	3rd instar	4th instar	5th instar
B1	19.65±1.04	16.95±1.64	19.87±0.37	9.83±0.14	38.29±1.98
B2	13.31±0.66	8.62±0.68	31.37±0.013	31.31±0.78	13.43±2.10
B3	7.38±0.13	4.38±0.26	Not visible	Not visible	7.22±0.19
B4	14.52±0.84	10.62±0.19	14.17±0.70	24.14±1.25	25.93±0.75
B5	Not visible	1.74±0.22	Not visible	2.78±0.30	Not visible
B6	5.96±0.06	2.64±0.015	Not visible	Not visible	Not visible

Table 5. Metrical analysis of mouthparts and its sensilla of different aged instars of *M, latifascia* (Mean length±SD micrometers)

Name of structures	1st instar	2nd instar	3rd instar	4th instar	5th instar
Distal segment of Mxp	16.00±0.78	21.98±0.20	14.04±1.56	45.23±4.45	16.46±2.61
Galea					
ST1	7.86±0.51	12.88±0.55	11.91±1.27	41.58±0.28	32.08±1.54
ST2	16.73±0.30	9.44±0.16	10.54±0.10	46.99±2.90	25.33±1.85
ST3	12.85±0.61	13.68±0.38	7.90±0.31	58.91±0.74	41.32±0.07
Sensilla of maxillary palp					
A1	3.18±0.15	3.66±0.37	3.90±0.21	3.69±0.20	3.05±0.25
A2	3.93±0.10	4.7±0.29	3.36±0.003	3.37±0.33	2.74±0.31
A3	4.30±0.39	4.17±0.54	4.33±0.19	4.06±0.15	3.47±0.31
L1	3.18±0.21	3.27±0.15	2.63±0.53	3.18±0.09	2.38±0.51
L2	2.26±0.26	3.37±0.40	1.66±0.07	2.24±0.01	1.88±0.10
L3	3.32±0.19	3.38±0.33	2.25±0.02	3.05±0.32	2.64±0.29
M1	1.42±0.05	3.58±0.29	1.86±0.02	3.02±0.05	1.80±0.13
M2	3.11±0.24	3.52±0.02	2.19±0.31	2.69±0.10	2.18±0.32
Sensilla of labial palp					
C	6.44±0.13	3.36±0.76	4.18±0.62	9.52±0.83	9.86±0.69
Sty	11.37±0.51	11.17±0.74	17.80±0.5	31.99±1.79	33.09±2.16

moth species, indicating potential differences in silk secretion and cocoon-spinning behaviour (Kaleka and Dulai 2024, Kaleka and Dulai 2025)..

The findings of this study underscore the importance of detailed ultrastructural analyses in lepidopteran taxonomy. The observed species-specific variations in sensilla distribution and morphology provide valuable insights into larval sensory adaptations, with implications for both phylogenetic studies and pest management strategies. Future research should focus on electrophysiological assessments to better understand the functional significance of these sensilla in the feeding and behavioural ecology of these species.

CONCLUSION

The presence of Sensillum B5 across all larval stages of *Artaxa vitellina* Kollar, in contrast to its absence in some instars of *Maeoproctis latifascia* Walker, indicates species-specific developmental trajectories and sensory adaptations. The consistent presence of sensilla campaniformia and sensilla digitiformia in *Artaxa vitellina* Kollar across all instars, versus their occurrence only in later instars of *Maeoproctis latifascia* Walker, highlights significant interspecific differences in sensory organ development and ecological adaptations.

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Survey and Water Quality Analysis of Springs in Kupwara Region of Kashmir Himalayas

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***Abstract*:** The present study describes water quality scenario and discharge studies of springs of Kupwara district in Kashmir Himalayas. The key parameters measured included pH, conductivity, calcium hardness, total hardness, nitrite, nitrate, ammonium, iron, phosphate, turbidity, salinity, total dissolved salts (TDS) and temperature. The pH levels ranged from 6.74 to 7.60, indicating generally neutral conditions. Conductivity varied from 215 to 380 µS, reflecting mineral content differences. Calcium hardness varied from 13 to 26 drops of reagent while total hardness ranged from 95 to 165 mgL^{-1}. Nitrite levels were consistently low with varying nitrate and ammonium levels. Iron concentrations were minimal, and phosphate levels ranged from 0.1 to 1 mgL^{-1}. Turbidity was low, indicating clear water with salinity levels ranging from 162 to 214.3 mgL^{-1}. Total dissolved salts varied between 49.6 ppm and 96.1 ppm. This study provides a comprehensive assessment of spring water discharge and quality in Kupwara, offering valuable insights for managing water resources and addressing potential climate change impacts on water availability.

Keywords: Water quality, Springs, Himalayas

In mountainous regions like Kupwara district in Jammu and Kashmir, India, springs are an important source of water for drinking, irrigation, and other domestic uses. The springs which are in thousands in Kashmir landscape have the potential to offer viable solution to the rising drinking water demand and therefore merit an attention for their protection and management (Bhat et al., 2022). Understanding the seasonal variations in spring discharge and their underlying causes is crucial for managing water resources effectively and ensuring water security for local communities. Seasonal fluctuations are a natural phenomenon that significantly influence various environmental and hydrological processes. The changes in the trend of precipitation, temperature and glacier melt are expected to impact the quantity and quality of spring water significantly (Panwar 2020). Kupwara district, situated in the scenic Kashmir Valley of India, offers a compelling case study for analyzing the interplay between seasonal changes and spring water availability, particularly concerning drinking water purposes. The district is endowed with a network of springs that serve as crucial freshwater sources for its population. These variations pose particular challenges for drinking water supply, as springs are often the primary source of potable water for rural communities, especially in hilly areas where surface water bodies are minimal (Prem et al., 2021). The importance of consistent spring discharge for drinking water cannot be overstated, given its role in supporting daily life and public health. The study comprises of the discharge of springs and the quality parameters of spring water. The outcomes will provide valuable insights for local authorities and stakeholders, facilitating the development of adaptive management strategies to address water supply challenges throughout the year.

MATERIAL AND METHODS

Study area: The Kashmir Valley is home to hundreds of springs that draw tourists and, by offering them a variety of experiences, significantly boost the local economy offerings. Kupwara district situated in the northernmost part of Kashmir valley is characterized by its unique topographical features, climatic conditions and rich hydrological sources .The district has an area of 2379 square kilometres .The longitude and latitude of district sprawls between 74° 35′ to 74° 45′ E longitude and 34° 15′ to 34° 45′ N latitude. Kupwara's temperate climate, marked by extreme cold winters and warm summers, introduces significant variability in the hydrological dynamics of these springs. Seasonal fluctuations impact the water table and discharge rates, leading to variable availability of spring water. Winters are typically harsh , lasting from mid-November to march with temperature dropping as low as -5^0C. The district receives an average annual precipitation of about 869nm. Kupwara geomorphology is marked by the presence of Pirpanjal mountain range which contributes to districts dramatic elevation changes .The region's topography plays a pivotal role in shaping its hydrological pattern including the formation

and discharge of springs. Our study area encompasses six springs situated across different locations in Kupwara district. These include Astannaag in Tirch Natnussa, Checkigam in Checkigaam, KVK Spring in Kupwara, Farrnaag in Kandi, Waninaag in Natnussa, and Shewaling in Drugmulla. Each spring represents a unique hydrological site, contributing to a diverse dataset for analyzing seasonal variations in spring water availability.

Sampling and water quality: Water samples were collected from the springs in three different months viz; January, April and June from six different locations viz; Astannaag in Tirch Natnussa, Checkigam in Checkigaam, KVK Spring in Kupwara, Farrnaag in Kandi, Waninaag in Natnussa, and Shewaling in Drugmulla in 1-liter plastic bottles. The physicochemical parameters *viz*; temperature, pH, total soluble solids (TSS),total dissolved salts (TDS), salinity, turbidity, conductivity, total hardness, calcium hardness, nitrite, nitrate, ammonium, iron and phosphate were examined for each sample (APHA 2005). Water samples were analyzed in a laboratory to determine their discharge rates and other relevant parameters. To measure the discharge rate of various spring outlets, a volumetric flask was used and stopwatch was used to estimate the time.

Table 1. Standard analytical methods adopted for different quality parameters

Parameters	Analytical techniques/ Methods	Units
pH	Electronic pH meter	------
TSS	Filtration method	Mg/L
TDS	Evaporation method	Ppm
Salinity	Salinometer/Conductivity meter	g/kg
Turbidity	Turbidity meter/Nephelometer	NTU
Conductivity	Electronic conductivity meter	mS
Total hardness	EDTA Titration method	Mg/L
Calcium hardness	EDTA Titration method	ppm
Nitrite	Open Colorimeter	ppm
Nitrate	Open Colorimeter	ppm
Ammonium	Open Colorimeter	ppm
Iron	Test kit	Mg/L
Phosphate	Test kit	Mg/L
Temperature	Digital Thermometer	^{0}C
Elevation	GPS Device	msl
Carbonates	Titration	Mg/L
Discharge	Volumetric flask	L/s

RESULTS AND DISCUSSION

Water quality analysis: The pH value ranged from 6.74 at 8.8 ^{0}C (Chekigam) to 7.55 at 7.6 ^{0}C (Farrnag) (Table 2). Most springs have a basic pH, which can be somewhat because of the limestone-rich geology of the Kashmir valley (Barakat *et al*., 2018). Conductivity ranged from a minimum of 272 µs(Waninag) to a maximum of 380µs (KVK Kupwara). The range of electrical conductivity (EC) indicate low to moderate mineral content /The concentration of Ca^{2+} and Mg^{2+} ions in water is the primary factor influencing its hardness. Hardness solely reflects the quality of the water; it is not a pollution indicator parameter. The overall range of 95 to 165 mg L^{-1} indicate that all of the springs' water is naturally hard. Calcium hardness was recorded at all six locations and ranged between a minimum of 110 ppm (moderately hard) (KVK Kupwara) to 180 ppm (hard) (Waninag). The WHO (World Health Organization) scale for calcium hardness in drinking water classifies water hardness based on the concentration of calcium and magnesium salts, which classifies it as soft water: 0 - 60 mg/L as $CaCO_3$; moderately hard water: 61 - 120 mg/L as $CaCO_3$; hard water: 121 - 180 mg/L as $CaCO_3$; Very hard water: > 180 mg/L as $CaCO_3$. Lacustrine deposits, which are sedimentary rocks like limestone, gypsum, and dolomite, are the source of Ca^{2+} and Mg^{2+}.

Nitrite content was absent in all springs studied except Astanaag (5 ppm). Nitrate content (5 ppm) was recorded in KVK Kupwara, Farnaag and Waninaag springs and zero in other springs. Ammonium concentration of 1 ppm in KVK Kupwara and Shewaling springs was recorded and 0.5 ppm in rest of the springs (Astanaag, Checkigam, Farrrnaag and Waninaag). Nitrogen in the form of nitrate (NO3 −), nitrite (NO2 −), and ammonia (NH3 +) present in natural water evoke great interest because of their nutrient values, thereby being limiting factors for many bio-chemical processes. Excessive fertiliser use and the addition of human and animal waste are the causes of a higher concentration of nitrate in groundwater (Mondal et al., 2008). Lower nitrate concentration was in Astanaag, Checkigam and Shewaling (0.00 ppm) because there are less agricultural activities in the

Table 2. Discharge rates of springs (l/s)

Month	Astannag	Checkigam	KVK Spring	Farrnaag	Waninaag	Shewaling
January	0.27	0.70	0.09	0.2 0	0.13	0.70
April	0.5 0	1.10	0.17	0.22	0.25	1.3 l
June	0.50	1.10	0.2 0	0.28	0.30	1.7 l

immediate catchment, however, KVK Kupwara, Farrnaag and Waninaag had nitrate concentrations of 5ppm because fertilizer sewage and organic matter decomposition is significant source of nitrates in spring water.

According to United States-, Environment Protection Agency (US- EPA), the recommended limit for iron content in portable water is 0.30 ppm., Waninaag and Shewaling had 0.3 mgL^{-1} iron content and 0.00 mgL^{-1} in Astanaag, Chekigam, KVK Kupwara and Farnaag which means that all the samples pass EPA recommendations . Apart from the organic matter enrichment, the iron (Fe) source could also be attributed to the iron-rich rocks found in the springs' catchment area (Rao 2007).

The concentration of phosphorus varied between 1 mg L^{-1} in Astanaag and Waninaag, 0.5 mg/L in Chekigam, KVK Kupwara and Farnaag and 0.1 mg L^{-1} in Shewaling. Phosphate sources can be man-made or anthropogenic, contingent on local human activity. In the catchment of these springs, where phosphate fertilisers are heavily used, there is convincing evidence of the direct influence of agricultural and horticultural activities due to the slightly greater concentration of phosphates in some springs (Kipngetich et al., 2013).

Turbidity values were almost non-significant in concentration differences. Because there was no surface disturbance, the springs exhibited low turbidity values. In the case of these springs, the lack of surface disruption meant that minimal external debris or pollutants were introduced into the water, resulting in clear and relatively undisturbed water quality. Additionally, the absence of rainfall or human activity, such as construction or agricultural runoff, likely prevented the introduction of particles that could cloud the water. Therefore, the stable, calm conditions allowed the springs to maintain their low turbidity levels, which is often a key indicator of cleaner, well-preserved water systems (Joshi and Agarwal 2022). Maximum salinity was 214.3 mg L^{-1} at 14.7 ^{0}C (KVK Kupwara) followed by Waninaag, Checkigam, Farrnaag. and Shewaling and minimum was 162 mg L^{-1} at 14.5 ^{0}C (Astanag). Salinity concentration is directly correlated with the chemical properties and geology of the aquifer (Naeem 2015).

The maximum total dissolved salts (TDS) was n KVK Kupwara (96.1 ppm at 25^0 C) and minimum was in Astanaag (49.6 ppm at 25 ^{0}C). Total dissolved salts in water originates from irrigation returns, urban runoff, natural sources, municipal, road deicing, industrial wastes, chemicals used in water treatment and the actual plumbing infrastructure (Hach solids. 2023). Drinking water with high concentrations of dissolved solids may affect the taste of water. In addition, high TDS levels can cause scaling and corrosion in any application, particularly in cooling water and boilers, and therefore need to be monitored in water systems (Adjovu et al., 2023).

Further it was observed that there was no presence of carbonates in any of the springs. Carbonates may be absent in some water samples because water may not have been in contact with carbonate rich rocks like limestone, meaning there no source to dissolve the carbonate minerals, or the

Table 3. Chemical analysis of various springs

Parameter	Astanaag	Chekigaam	KVK Kupwara	Farrnaag	Waninaag	Shewaling
pH	7.O3 at 6.8^0 C	6.74 at 8^0 C	7.17 at 7.6^0 C	7.55 at 7^{0}C	7.43 at 7^{0}C	7.35 at 7.3
Conductivity	215 µs	332 µs	380 µs	282 µs	272 µs	290 µs
Calcium hardness	130 ppm	125 ppm	110 ppm	150 ppm	180 ppm	165 ppm
Total hardness	135 mg L^{-1}	110 mg L^{-1}	95 mg L^{-1}	145 mg L^{-1}	165 mg L^{-1}	150 mg L^{-1}
Nitrite	5 ppm	0.0 ppm	0.0 ppm	0.0 ppm	0.0 ppm	0.0 ppm
Nitrate	0.0 ppm	0.0 ppm	5 ppm	5 ppm	5 ppm	0.0 ppm
Ammonium	0.5 ppm	0.5 ppm	1 ppm	0.5 ppm	0.5 ppm	1 ppm
Iron	0.00 mg L^{-1}	0.0 mg L^{-1}	0.00 mg L^{-1}	0.0 mg L^{-1}	0.3 mg L^{-1}	0.3 mg L^{-1}
Phosphate	1 mg L^{-1}	0.5 mg L^{-1}	0.5 mg L^{-1}	0.5 mg L^{-1}	1 mg L^{-1}	0.1 mg L^{-1}
Turbidity	004 NTU At 12.2^0 C	005 NTU At 12.3^0 C	006 NTU At 11.9^0 C	006 NTU At 11.9 ^{0}C	006 NTU At 12.9^0 C	006 NTU At 12^0 C
Salinity	162 Mg/L at 14.5 ^{0}C	185.6 Mg/L at 14.8^0 C	214.3 Mg/L at 14.7^0 C	183.8 mg L^{-1} at 14.8 ^{0}C	194.7 mg L^{-1} at 14.8 ^{0}C	174.6 mg L^{-1} at 14.4^0 C
Total Dissolved Solids	49.6 ppm at 25^0 C	54.4 ppm at 25^0 C	96.1 ppm at 25^0 C	62.9 ppm at 25^0	55.8 ppm at 25 ^{0}C	70 ppm at 25 0C
Elevation	1689 msl	1706 msl	1591 msl	1682 msl	1623 msl	1680 msl
Temperature	10. 3^0 C	9.9^0 C	11.8^0 C	10.5^0 C	9.7 ^{0}C	11.3 ^{0}C
Carbonates	Absent	Absent	Absent	Absent	Absent	Absent

water chemistry could be too acidic, preventing carbonate ions from forming and remaining stable in solution (Shmeis 2018).

Discharge of springs

The discharge values of springs was studied in January, April and June so as to know about variations in discharge due to different seasons. The values of discharge rates in KVK Kupwara spring was 0.08, 0.17 and 0.2 l/s in January, April and June, respectively. Similarly, the discharge values of Checkigam (0.7, 1.0 and 1.0 l/s), Astanag (0.27, 0.5 and 0.5 l/s), Shewaling (0.7, 1.3 and 1.7 l/s), Waninaag (0.13, 0.25 and 0.3 l/s) and Farnag (0.2, 0.22 and 0.28 l/s) in January, April and June, respectively. The discharge in springs varies seasonally and is influenced by hydro-geological conditions, climate change and anthropogenic factors like ground water abstraction (Casati *et al*. 2024). The relationship between precipitation and spring discharge is influenced by seasonal changes in high altitude recharge areas. From December to March, precipitation accumulates as snow, resulting in minimal ground water recharge due to frozen conditions. A temperatures rise in April, snow melt begins, infiltrating the ground and increasing spring discharge. After June the discharge is seen to be maximum in all the springs (Chang at al., 2021) (Gao et al., 2016).

CONCLUSIONS

This study provides a comprehensive analysis of spring discharge and water quality in the Kupwara District. Studies were conducted on the differences in the water quality analysis and discharge rates of 06 springs located throughout the district. The measured discharge rates showed variability among springs but did not significantly change across different seasons, indicating stable hydrological conditions. Water quality assessments revealed generally hard water with pH values leaning towards basic, attributed to the region's limestone geology. It is crucial to implement sustainable water management practices to safeguard springs from depletion and contamination. This involves regulating land use changes and reducing deforestation in catchment areas, as these activities can interfere with the natural hydrology.

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Effect of *Trigonella foenum* Gracum (Fenugreek Seed) on Growth and Survival of *Labeo Rohita*

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Abstract: A 60-day feeding trial was evaluated to assess the effects of fenugreek (*Trigonella foenum-graecum*) on the growth, and survival of Rohu (*Labeo rohita*). There were five treatment groups, with three replications. The fish were fed five different inclusion levels of Fenugreek seed (FS) in their diet i.e., without fenugreek seed and with 0.5, 1, 1.5 and 2% Fenugreek seed). The protein level in all the diets was consistently maintained at approximately 30%. *Labeo rohita* were stocked at 10 nos. /tank density in all experiment tanks. There were significant difference in growth parameters among treatment groups. Highest weight gain , specific growth rate , protein efficiency ratio and a lower feed conversion ratio was observed 1% fenugreek seed treatment and also recorded better survival rates than the control. The study recommends including 1%fFenugreek seed in *L. rohita* diets for enhanced growth and survival.

Keywords: *Trogonella foenum graecum*, *Labeo rohita*, Growth parameters, Fenugreek seed

The fisheries sector is crucial in the global food economy, providing essential food and nutritional security, especially for developing and underdeveloped countries. Aquaculture is rapidly evolving into a key sector in the agricultural economy, propelled by the growing need for affordable, high-quality animal protein, particularly as the global population continues to rise (Rathore and Swain 2024). Aquaculture is one of the fastest-growing sectors within India's fisheries industry, with an annual growth rate exceeding 7%. Freshwater aquaculture accounts for nearly 80% of the country's inland fish production. Focusing on juvenile and exotic carp species, India has become the second-largest producer of farmed fish globally (FAO 2024).

In India, carp culture is the cornerstone of freshwater aquaculture and aligns well with other farming systems. Its compatibility with diverse agricultural practices makes it crucial for sustainable aquaculture, supporting food security and rural livelihoods across the country. *Labeo rohita*, a species that is extensively distributed across India and South Asia, plays a crucial role in enhancing overall productivity in aquaculture. Its adaptability to various environmental conditions and compatibility with other species make it an essential component of polyculture systems, where it helps optimize resource utilization and improve the economic viability of aquaculture operations across the region. Rohu is economically significant due to substantially higher muscle protein content compared to other carp species and strong consumer demand (Shakir et al., 2013). *Trigonella foenum-graecum*, commonly known as fenugreek have high protein and additionally, fenugreek seeds contain a variety of bioactive compounds, including flavonoids such as quercetin, apigenin, and kaempferol, as well as saponins like graecunin, fenugrin B and fenugreekine. This study was designed to investigate the effects of natural feed additives fenugreek seed (*Trigonella foenum-graecum*) on growth performance and survival rates of *Labeo rohita*.

MATERIAL AND METHODS

Experiment setup: The research was carried out on *Labeo rohita* as the experimental animal at College of Fisheries Science, Kamdhenu University, Veraval. (20° 93' 92.54" N, 70° 35' 26.79" E). The seeds of *L. rohita* were transported from the nearby fish commercial hatchery in an oxygenated polythene bag. Upon the arrival of the seeds, they were acclimated in a 500 L capacity fiber-reinforced plastic (FRP) tank for 15 days. Continuous aeration and feeding were provided during the acclimatization period. A total of 200 fish were used in the experiment. They were fed at the rate of 5-7% of their body weight, and optimum water quality and adequate oxygen levels were maintained during the experimental period.

Twenty tanks were used, each with a capacity of 50 litters and dimensions of 2×1×1 feet. The experimental design was completely randomized design (CRD). The tanks were divided into five different treatment groups, with each treatment being replicated three times. After a period of acclimatization, the fish were introduced into the plastic aquaria tanks, where they were kept for 60 days. Each tank was stocked with 10 fish and supplied with sufficient aeration.

Experimental diet: For this experiment, fenugreek seed were collected from a local market of the Veraval. The seed were ground into a fine powder and stored in a dry place.

Additional ingredients like fish meal, soya bean meal, wheat flour, tapioca flour, and plant oil were sourced from local markets to maintain quality and accessibility. All ingredients were ground and sieved for uniformity, stored in polythene bags at room temperature.

Five experimental diets were prepared, each containing 30% protein. The control diet, labelled T0, did not include fenugreek seed. The diets, labelled T1, T2, T3, and T4, included fenugreek seed (FS) at concentrations of 0.5, 1, 1.5, and 2%, respectively. The ingredients were weighed, ground, sieved, and thoroughly mixed. Prepared feed was carefully stored in airtight containers (Table 1).

Measurement of growth and survival: At the end of the 60-day experimental period weight and survival rate were recorded. The growth parameters such as mean weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated.

Mean weight gain = Final average body weight - initial average body weight

Specific growth rate (%) = [(ln final weight – ln initial weight) / Experimental period (day)] × 100

Feed conversion ratio = Feed intake (g) / wet weight gain (g)

Protein efficiency ratio = Weight gain (g) / protein intake (g)

Survival (%) = (No. of fish survival at the end of the experiment/no. of fish stocked) ×100

Analysis of physic-chemical water quality parameters: Throughout the experiment period, physio-chemical parameters including temperature, dissolved oxygen (DO), pH and alkalinity were monitored weekly. The DO levels in the water samples were determined using the Winkler method (Fi et al., 2014). Alkalinity was measured using the EDTA method (Summerfelt 2000). Temperature was recorded by thermometer and pH was measured using a standard digital pH meter and pH indicator solution (Chun et al., 2018).

Statistical analysis: The statistical analysis was performed using SPSS software and excel with descriptive values Duncan's Multiple Range Test (DMRT) was used to compare the mean value.

RESULTS AND DISCUSSION

Mean weight and mean weight gain: The initial average weights of Rohu varied from 1.40 to 1.42 g with non-significant differences. At the end of the trial, the final mean weights of Rohu in the was significantly higher in 1% fenugreek seed followed by 0.5, 1.5 and 2% incorporation level (Table 2). Survival rates were recorded 95, 92.50, 91.25, 90.00 and 88.75% for treatments T2, T1, T3, T4, and T0 (Table 2). Mehboob et al., (2017) observed maximum survival rates in the diet containing 1% fenugreek seed compared to the control group. Similar results were reported by Rathore et al., (2018), Moustafa et al., (2020), and Roohi et al., (2015). Survival is crucial in fish production, influenced by feed availability, water quality and other environmental factors. Fish survival rates were consistently high between 88.75% to 95.00%. Higher survival was observed in T1 which had 1% Fenugreek seed. Syeed et al., (2018) observed that the diet of common carp containing 1% fenugreek had higher survival rate.

Specific growth rate: Specific growth rate (SGR) in control treatment T0, which lacked fenugreek, exhibited the lowest SGR among all treatments. The highest SGR was in T2 (2.01%) treatment. Rathore et al. (2018). Kumar et al. (2017) and Moustafa et al. (2020) also observed similar result in 1% inclusion level.

Feed conversion ratio: The lowest FCR was observed in treatment T2 (2.087), followed by T1, T3, T4 and T0 (2.336) (Table 3). There was a significant difference in the FCR across the different treatments performed. Treatment T2 demonstrated a significantly better feed conversion ratio when compared to all other treatments as well as the control group. Mehboob et al. (2017) reported that FCR decreased in

Table 1. Ingredients and their proportion in experimental diets

Ingredients (%)	Diets (30% protein)				
	T0 (0 %)	T1 (0.5 %)	T2 (1 %)	T3 (1.5 %)	T4 (2%)
Fenugreek seed	0g/kg	5g/kg	10g/kg	15g/kg	20g/kg
Fish meal	30	30	30	30	30
Soybean meal	22	19	15	14	13
Wheat flour	25	23	22	18	14
Tapioca flour	12	12	12	12	12
Plant oil	2	2	2	2	2
Fish oil	2	2	2	2	2
Vitamins and minerals	2	2	2	2	2

Table 2. Growth performance and survival of *Labeo rohita* during the experiment (Mean ± standard error)

Treatment	Mean weight (g)				Survival (%)
	Initial	30th Day	45th Day	60th Day	
T0	1.40[a]	2.58±0.014[c]	3.17±0.010[d]	3.83±0.011[d]	88.75±1.250[b]
T1	1.42[a]	2.86±0.017[b]	3.64±0.020[b]	4.45±0.023[b]	92.50±1.443[ab]
T2	1.41[a]	2.97±0.019[a]	3.81±0.013[a]	4.70±0.011[a]	95.00±2.041[a]
T3	1.41[a]	2.84±0.013[b]	3.62±0.015[b]	4.43±0.033[b]	91.25±1.250[ab]
T4	1.40[a]	2.60±0.015[c]	3.34±0.012[c]	4.05±0.015[c]	90.00±2.041[ab]

Figures with same letter in column indicate non-significant differences

Table 3. Specific growth rate, food conversion ratio and protein efficiency ratio of *Labro rohita* (Mean ± standard error)

Treatment	Specific growth rate (SGR)	Feed conversion ratio (FCR)	Protein efficiency ratio (PER)
T0	1.67±0.014[d]	2.692±0.086[c]	1.064±0.068[c]
T1	1.91±0.017[b]	2.285±0.036[ab]	1.251±0.039[b]
T2	2.01±0.026[a]	2.087±0.019[a]	1.369±0.024[a]
T3	1.90±0.017[b]	2.336±0.069[ab]	1.226±0.069[b]
T4	1.77±0.015[c]	2.485±0.134[bc]	1.160±0.127[bc]

Figures with same letter in column indicate non-significant differences

the diet containing 1% FS in striped catfish, *Pangasius hypophthalmus*. Roohi et al., (2015) also reported similar findings in *Cyprinus carpio*.

Protein efficiency ratio: The maximum PER was 1.369 in T2 followed by T1, T3, T4 and minimum in control (1.064) (Table 3). There was no significant difference in the protein efficiency ratio between the treatment T1 and T3 compared to treatments T4 and T0 but were to be at par with T2. The PER of was investigated by Rathore et al. (2018) observed that a diet containing 1% FS had a good PER in *Nile tilapia*. Similar trend was observed by Syeed et al. (2018), Mehboob et al. (2017) and Moustafa et al. (2020).

Specific growth rate: The specific growth rate (SGR) was significantly higher in T2 ((2.01%- 1% fenugreek), while treatment T0 showed the lowest SGR. There was no significant differences between treatments T1 and T3. Treatment T4 had a higher SGR than T0 but lower than treatment T1 and T3. Syeed et al., (2018), found that the highest growth performance was observed in the group, fed a diet with 1% fenugreek, while the control group had the lowest growth. Mehboob et al. (2017) observed a significant difference among the three treatments. In 1% FS group had a significantly higher mean weight as compared to T1 (0.5% FS) and the control. Roohi et al. (2015) also found similar trend in *Cyprinus carpio*. The fish-fed diets with fenugreek had significantly more weight gain compared to the control group diosgenin, a compound commonly used in steroid production, this may increase weight gain.

In the present investigation, treatment T2 (2.08) exhibited the best feed conversion ratio, followed by treatment T1, T3, and T4. Fish fed a diet containing 1% fenugreek seed indicated best feed conversion ratio. This means they needed less food to gain weight compared to other groups. Fenugreek seeds are particularly high in protein and are also a significant source of diosgenin, which might increase the growth of fish. Fenugreek seeds are rich in non-starch polysaccharide (NSP) fibers, with the major NSPs being tannins, saponins, hemicelluloses, mucilages, and galactomannans. These non-starch polysaccharides promote bowel movements, aid in smooth digestion and help to reduce blood cholesterol levels by binding to it (Riaz et al., 2020).

CONCLUSION

A 60-day feeding trial was conducted to evaluate the effects of fenugreek (*Trigonella foenum-graecum*) seed on the growth and survival of *Labeo rohita*. Five treatment diets with varying fenugreek seed levels (0 to 2%) were formulated, maintaining a protein level of 30%. The diet with 1% fenugreek seed showed the highest growth performance, including mean weight gain , specific growth rate, protein efficiency ratio and lowest feed conversion ratio along with the highest survival rate. The study recommends incorporating 1% fenugreek seed in *L. rohita* diets to enhance growth and survival, supporting sustainable aquaculture practices.

AUTHOR'S CONTRIBUTION

Honey J. Tandel and Rajesh V. Chudasama conducted the data collection, performed the analysis, and wrote the

manuscript. Hitesh V. Parmar reviewed the manuscript and provided guidance throughout the study. Binal Tandel contributed to the manuscript by making corrections and ensuring clarity.

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Effect of Low-Temperature Preservation on Growth Characteristics of Marine Microalga *Nannochloropsis salina*

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***Abstract*:** The present study aimed to preserve the microalgae, *Nannochloropsis salina* at three different temperatures (0, -4 and -20℃) following standard protocols and assess their viability and growth characteristics upon thawing. The algal cells were preserved in Conway medium following seven different treatments: 0℃ without addition of cryoprotectant, -4℃ without cryoprotectant, -4℃ with 5 and 10% Me_2SO and - 20℃ with 5 and 10% Me_2SO along with a control at room temperature (24℃) for 5 days. Upon air thawing, the cells were sub-cultured at optimum conditions and were analyzed for the growth characteristics, viz., biomass, specific growth rate, cell density and optical density for 5 days. Among the treatments, the sample preserved at -4℃ without addition of any cryoprotectant exhibited better performance in terms of biomass (0.0165 g/ml), optical density (0.0265) and cell density (4.78 x 10^5 cells/ml). Similarly, the cells preserved at -20℃ with 10% cryoprotectant exhibited poor growth performance in terms of cell density (1.86 x 10^5 cells/ml) and biomass (0.0085 g/ml) which may be attributed to the toxicity of cryoprotectants. Thus, *Nannochloropsis salina* can be preserved at -4℃ without adding any cryoprotectant. This eliminates the need to maintain continuous sub-cultures in aqua hatcheries, which is laborious and costly.

***Keywords*:** Cryoprotectant toxicity, Thawing, Dimethyl sulphoxide, Conway medium, Microalgal culture

Aquaculture, the fastest-growing food production sector, heavily relies on microalgae due to its nutritional benefits, particularly for larval stages of fish, molluscs, and crustaceans in hatcheries. Key microalgae used in aquaculture include *Nannochloropsis* sp., *Chlorella* sp., *Chaetoceros* sp., *Tetraselmis* sp., *Scenedesmus* sp., *Pavlova* sp., *Phaeodactylum* sp., *Skeletonema* sp., and *Thalassiosira* sp.(Hemaiswarya et al., 2011 , Sirakov et al., 2015). Among these, *Nannochloropsis* spp. stands out for its small size (2-4 µm diameter, subspherical or cylindrical), high growth rates, and resilience to environmental fluctuations in temperature, light, and nutrient levels. *Nannochloropsis* spp. belongs to the group, Eustigmatophyceae and are highly valued in aquaculture for their nutritional profile, containing 43% protein, 16.6% lipid, and high levels of eicosapentaenoic acid (EPA), which constitutes 16% of the total lipid content (Guimarães et al., 2021). In addition to its nutritional benefits, *Nannochloropsis* spp. exhibit high photosynthetic efficiency and a digestible cell wall composed of simple polysaccharides (63 to 119 nm in thickness) and are rich in pigments like chlorophyll, zeaxanthin, canthaxanthin, and astaxanthin (Shah et al., 2018). These properties make *Nannochloropsis* spp. an ideal candidate as live feed and for enriching zooplankton, which can further enhance the growth of aquaculture species (Siddiqui et al., 2024). Algae, both micro and macroalgae, are also known for their rich source of bioactive compounds for biotechnological interventions (Siddhnath and Kaur 2023).

The feeding of larval stages of aquatic organisms in hatcheries heavily relies on a steady supply of microalgal starter cultures, which can be sourced from commercial suppliers, government labs, universities, or directly from other hatcheries. Obtaining starter cultures from other working hatcheries is common, as many operators willingly share cultures with fellow culturists. Once acquired, these starter cultures are used to inoculate new cultures, creating a stock culture that is then serially sub-cultured. However, this conventional method demands significant space, substantial growth media, and incurs high labor costs. Additionally, it poses ongoing challenges with contamination and genetic drift, which may alter the desired characteristics of the algal strains over repeated sub-culturing (Helliwell et al., 2011, Berge et al., 2012). An effective alternative is low-temperature preservation. Over the past years, different protocols and cryoprotective additives (CPAs) have been tested in order to increase the post-thaw survival rate of microalgae (Abreu et al., 2012, Chellappan et al., 2020). There is no universal protocol for the cryopreservation of algae, which is not surprising considering the substantial heterogeneity in morphology, physiology, and ecology. Hatcheries generally have access to freezer storage for chemicals and feeds, making low-temperature storage an accessible option. This study, therefore, aims to develop a cost-effective method to store algal cultures at low temperatures, allowing hatcheries to thaw and sub-culture algae on demand, reducing resource use and preserving the integrity of algal strains.

MATERIAL AND METHODS

Species for study: The microalgal species used for the study, *Nannochloropsis salina* was obtained from the algal culture laboratory, Seabass Hatchery Unit, Rajiv Gandhi Centre for Aquaculture, Sirkali. Conway medium was used for the invitro culture of *Nannochloropsis salina* (Table 1). All glassware used in the study were sterilized in autoclave at 121℃ (15 lbs pressure) for 15 minutes. Ten milliliters of collected samples were transferred aseptically to the conical flasks (ml) containing the respective growth media. The experiments were performed at 22°C and the cultures were illuminated underneath by cool-white fluorescent lamps with a Photosynthetically Active Radiation (PAR) of 50 µmol photons $m^{-2}s^{-1}$ under a 12L: 12D regime in growth media prepared using 33 ppt artificial seawater (Table 2). The distilled water was autoclaved at 121°C for 15 minutes, prior to the preparation of artificial seawater to avoid contamination. The cultures were manually shaken twice daily as no agitation through aeration was provided. The culture was used as an inoculum and stock culture for further studies.

Preservation of the microalgal cells: Dimethyl sulfoxide (Me_2SO) was used as cryoprotectant in this study. The cryoprotectant was mixed with distilled water at twice the desired final concentration. For preparing 5% Me_2SO, 10 ml of Me_2SO was mixed with 10 ml of water and finally made up to 100 ml. In the same way 10% Me_2SO solution was prepared.

Preservation at 0°C: For preservation of the sample at 0°C, 2ml of stock culture without any cryoprotectant was taken in vials in duplicate and were stored in refrigerator for 5days. The preservation of samples at -4°C was carried out with and without the addition of cryoprotectant. One portion of the sample was stored at -4℃ without the addition of any cryoprotectant. For preservation using cryoprotectant, 0.1 ml of 5% Me_2SO was added with 1.9 ml of stock culture in 2 vials and 0.1 ml of 10% Me_2SO was added with 1.9 ml of stock culture in separate vials. All the vials were stored at -4°C for 5days. For preservation at -20°C, 0.1 ml of 5% Me_2SO along with 1.9 ml of stock culture taken in 2 vials and 0.1 ml of 10% Me_2SO was added with 1.9 ml of stock culture in separate vials. The vials were rapid frozen using 'Mr. Frosty' Freezing container (Nalgene®, Nalge Nunc International) containing isopropyl alcohol, previously cooled to 4°C, at a freezing rate of-1℃ min. The frozen vials were stored at -20°C for 5 days.

Thawing and post-thaw evaluation: After 5 days of storage, the vials were taken and air-thawed at room temperature (24°C). After thawing, the contents of the vials were diluted by stepwise addition of 100 ml of growth medium (Conway medium) and the cultures were incubated at 24°C (at 1000 lux) along with control (stock culture) and their growth characteristics viz., biomass, cell density, Specific Growth Rate and optical density (OD) were analyzed daily for 5 days.

Biomass: Biomass was estimated using 2 ml microalgal samples filtered through pre-combusted (100°C, 4 h) and pre-weighed glass fibre filters (Advantec, Japan). After filtration, algal samples were rinsed with 2 ml of 0.5 M ammonium formate. The filtrates were dried at 100°C for 4 h, cooled in a desiccator and then weighed. The dry biomass concentration in the culture was calculated by dividing the difference between the weights of the dried filter paper (after and before filtration) by the filtered volume (Lavens and Sorgeloos 1996).

Cell density: Cell numbers were determined daily by placing an aliquot of well-mixed culture suspension on a hemocytometer (Neubauer Improved Assistant, Germany).

Table1. Chemical composition of Conway medium

Chemical name	Quantity (g/L)
Solution A	
Potassium nitrate(KNO_3)	10
Sodium Di-hydrogen orthophosphate ($NaH_2PO_4.2H_2O$)	2
EDT Adi sodium salt(Na_2EDTA)	4.5
Boric acid (H_3BO_3)	3.34
Ferric chloride($FeCl_3$)	0.13
Manganese chloride($MnCl_2$)	0.036
Solution B	
Zinc chloride($ZnCl_2$)	0.42
Cobalt chloride ($CoCl_2.6H_2O$)	0.4
Copper sulphate ($CuSo_4.5H_2O$)	0.4
Ammonium molybdate	0.18
Con. Hydrochloric acid	2ml
Solution C	
VitaminB_1	0.002
Vitamin B_{12}	0.001

To prepare Conway medium, 1ml of solution A, 0.5 ml of solution B and 0.1 ml of solution C were mixed with 1 litre of seawater

Table 2. Chemical composition of artificial seawater

Compound	Quantity (g/L)
Sodium chloride (NaCl)	28.32
Magnesium chloride hexahydrate ($MgCl_2.6H_2O$)	5.48
Magnesium sulphate($MgSo_4$)	3.60
Calcium chloride ($CaCl_2.2H_2O$)	1.11
Potassium chloride (KCl)	0.77
Sodium bicarbonate	0.18

The cell number in the culture was calculated by dividing the number of cells counted by the volume and the dilution. The cell growth was measured by Neubauer counting chamber and the total number of cells were calculated using the following formula:

Total cell count (per ml) = Average no. of cells in each chamber x 10^4

Optical density: The optical density (OD) values for all cultures were determined daily using a UV-Vis spectrophotometer (Systronics, India). The wavelength used was 680 nm.

Specific growth rate: The specific growth rate (SGR) of microalgae was calculated by the following equation:

$$SGR/day = \ln (X_2/X_1)/t_2\text{-}t_1$$

X_1 is the biomass concentration at the beginning of the selected time interval;

X_2 is biomass concentration at the end of the selected time interval;

t_2-t_1 is the selected time (in days) for the determination of biomass of microalgal species.

Statistical analysis: Analysis of Variance (ANOVA) and the post-hoc analysis using Tukey's Honestly Significant Difference (HSD) was done using SPSS version 26.

RESULTS AND DISCUSSION

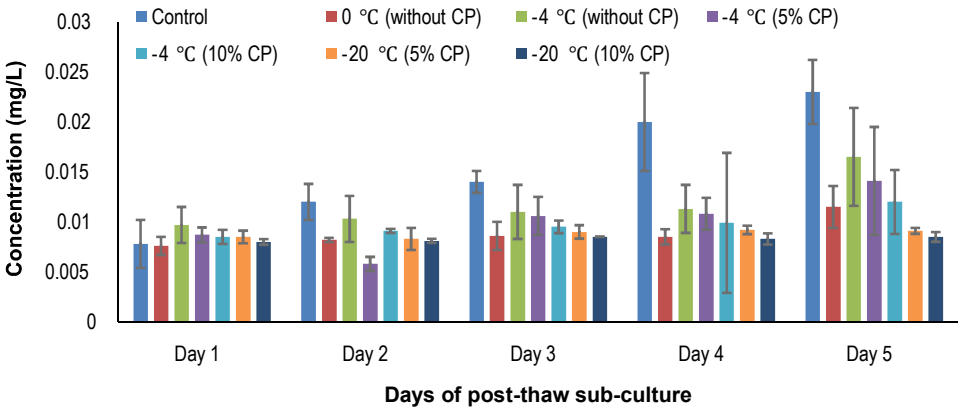
Biomass and specific growth rate: The increase in biomass observed across all treatments with continued sub-culturing suggests that the microalgal cells remained viable after low-temperature preservation and successfully resumed growth post-thawing (Fig. 1). This rise in biomass is indicative of the cells' ability to recover and proliferate following cryopreservation. After five days, the sample preserved at -4°C without any cryoprotectant demonstrated significantly highest biomass concentration (0.0165 g/L), which suggests that the absence of cryoprotectant might have mitigated potential stressors, allowing for optimal recovery and growth followed by biomass in cells preserved at -4°C with 5% cryoprotectant (0.141 g/mL). Similarly, the cells preserved at -4°C exhibited the highest specific growth rate (0.003 - 0.005 g/mL/day) at the 4th and 5th days of post-thaw sub culture (Fig. 2). This result implies that a moderate cryoprotectant concentration may have supported cell viability while reducing damage from freeze-thaw cycles, aligning with findings from previous studies that suggest a balanced cryoprotectant concentration is often beneficial for post-thaw viability and growth (Hubálek 2003). The lowest biomass concentration was observed in cells preserved at -20°C with 5% cryoprotectant (0.0085 g/mL) which may be due to increased cryoprotectant toxicity and potential osmotic stress, particularly at higher concentrations. Chellappan et al. (2020) also reported that elevated cryoprotectant levels could reduce post-preservation growth rates, as evidenced in diatoms that showed higher growth rates after storage with reduced cryoprotectant exposure in liquid nitrogen vapor phase.

Optical density: The differences in optical density (OD) among the samples preserved under various conditions highlight the impact of cryoprotectant concentration on cell preservation and, consequently, biomass retention (Fig. 3). The highest OD was in the sample preserved at -4°C without any cryoprotectant (0.0265) indicates that the absence of cryoprotectant may have minimized potential osmotic or metabolic stress on the cells. This preservation method possibly allowed for better maintenance of cellular integrity, leading to higher biomass retention as seen in the OD measurement. This was followed by samples preserved with 5% cryoprotectant at -4°C (0.0255). This suggests that a moderate cryoprotectant concentration might offer a balance between protecting cells from freeze-thaw damage and minimizing osmotic stress (Prieto-Guevara et al., 2023). Cryoprotectants are known to stabilize cell membranes and protect intracellular components, yet higher concentrations can sometimes induce osmotic imbalances or stress, which could potentially harm cells. Moreover, Me_2SO, usually, at a

Table 3. Variations in the cell density of *Nannochloropsis salina* upon thawing and sub-culturing

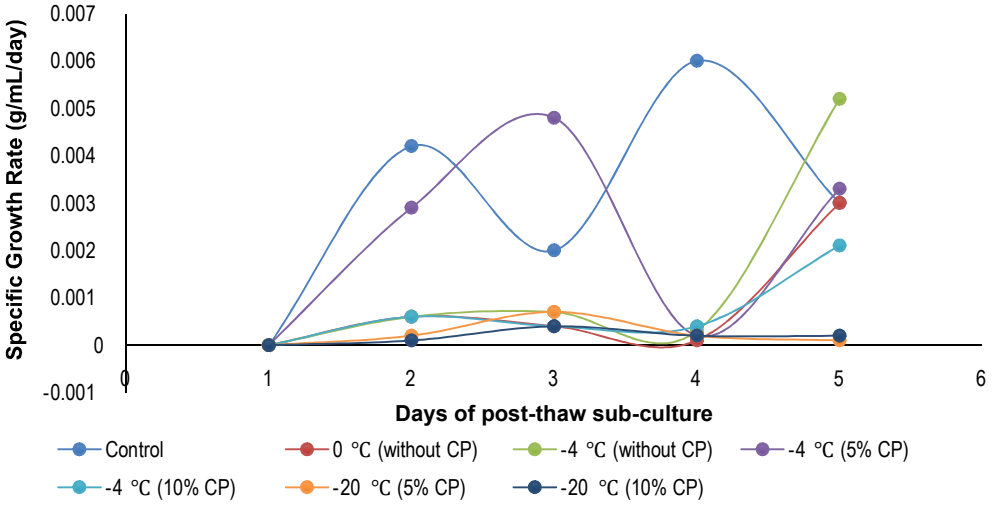
Treatment	Cell density (x10^5 cells/ml)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Control	2.02 ±0.016^{b}	2.14±0.0014^{bc}	2.56±0.02^{cd}	2.98±0.022^{b}	3.52±0.024^{bc}
0℃ (without CP)	1.65±0.002^{b}	1.97±0.004^{c}	2.22±0.0055^{cd}	2.55±0.0065^{bc}	2.58±0.01^{c}
-4℃ (without CP)	2.22±0.012^{b}	3.83±0.0155^{a}	4.47±0.0195^{a}	4.7±0.021^{a}	4.78±0.0265^{a}
-4℃ (5% CP)	3.27±0.075^{a}	3.11±0.0038^{ab}	3.75±0.015^{ab}	4.16±0.0175^{a}	4.19±0.0255^{ab}
-4℃ (10% CP)	2.55±0.055^{ab}	2.67±0.0075^{b}	2.95±0.0014^{bc}	3.35±0.0124^{b}	3.43±0.0028^{bc}
-20℃ (5% CP)	1.98±0.004^{b}	2.22±0.0044^{bc}	2.45±0.007^{cd}	2.6±0.008^{bc}	2.7±0.012^{c}
-20℃ (10% CP)	1.57±0.0014^{b}	1.71±0.0035^{c}	1.8±0.0035^{d}	1.82±0.0045^{c}	1.86±0.008^{c}

The values are expressed in Mean ± Standard deviation. Mean values having different superscripts on the same column are statistically different ($p < 0.05$)



The values are expressed in Mean ± Standard deviation. Error bars indicate standard deviation Mean values having different superscripts on the same day are statistically different ($p < 0.05$). The percentages in the paratheses of the legend represent the concentration of cryoprotectant used in the treatment

Fig. 1. Variations in the biomass of *Nannochloropsis salina* upon thawing and sub-culture



The percentages in the paratheses of the legend represent the concentration of cryoprotectant used in the treatment

Fig. 2. Variations in the post-thaw specific growth rate of *Nannochloropsis salina* subjected to various storage temperatures

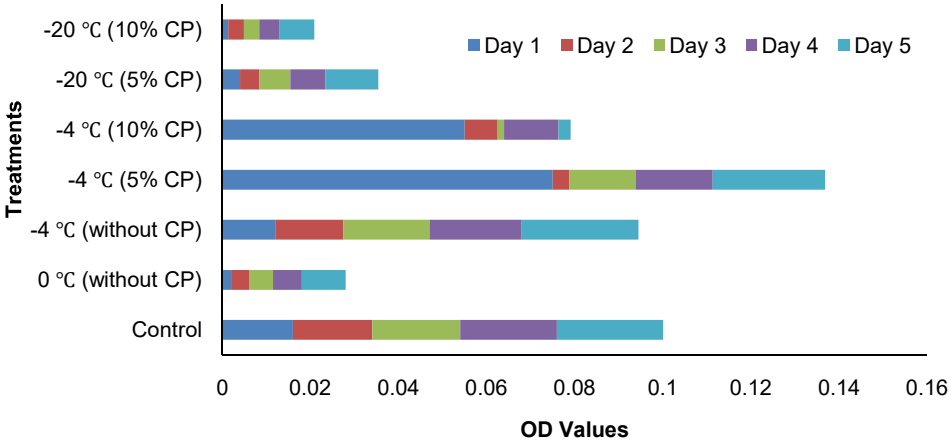


Fig. 3. Variations in the optical density of *Nannochloropsissalina* upon thawing and sub-culture

concentration of 5-10% has proved to be the most effective cryoprotective agent for a wide range of microalgal classes due to its low molecular weight, permeability, and free radical scavenging activity (Thomashow 1999). The reduced OD with 10% cryoprotectant likely reflects such a stress response, possibly leading to compromised cell integrity and reduced biomass retention. The lowest OD observed in the *Nannochloropsis salina* preserved with 10% cryoprotectant implies that this higher concentration may have negatively affected cell viability. *Nannochloropsis salina* might be sensitive to higher cryoprotectant concentrations, which could lead to cell shrinkage, membrane damage, or other structural issues. This is in alignment with the lower biomass values obtained, suggesting that while cryoprotectants provide a degree of protection, the concentration must be carefully optimized. *Nannochloropsis* sp. and other microalgal species have also indicated that low to moderate cryoprotectant concentrations (typically 5%) often preserve cells effectively by reducing ice-induced mechanical damage without introducing excessive osmotic stress (Abreu et al 2012).

Cell density: The cell density of the samples increases with the days of sub-culture. The highest cell density (4.78×10^5 cells/ml) was observed in samples preserved at -4°C without cryoprotectant, with statistical significance. The absence of cryoprotectants can sometimes be favourable, as cryoprotectants, while protective, may introduce osmotic or toxic stresses at certain concentrations (Hubálek 2003). The cells in this treatment were able to resume growth rapidly, leading to high post-thaw cell densities. The followed by in samples preserved at -4°C with 5% cryoprotectant. This treatment likely balanced the protective benefits of the cryoprotectant with minimal osmotic stress, promoting cell recovery and growth, as reflected in consistent biomass and OD values (Abishag et al 2025). The lowest cell density was recorded in samples preserved at -20°C with 10% cryoprotectant (1.86×10^5 cells/ml). This lower density may be due to the toxicity or osmotic stress introduced by the higher cryoprotectant concentration and the harsher preservation temperature, which can lead to membrane disruption and reduced cell recovery rates (Prieto-Guevara et al., 2023). Higher concentrations of cryoprotectants can create hyperosmotic environments, which may harm cells, especially at lower temperatures where cryoprotectant toxicity can be exacerbated. The findings highlight the importance of optimizing cryoprotectant concentration and storage temperature to ensure high post-thaw viability and recovery in microalgal cultures, particularly in species sensitive to osmotic stress.

CONCLUSION

The results demonstrate that *Nannochloropsis salina* preserved at -4°C without any cryoprotectant exhibited superior growth performance, as indicated by higher biomass, cell density, and optical density values. In contrast, cells preserved at -20°C with 10% cryoprotectant showed poorer growth performance, likely due to the cryoprotectant's toxicity at this concentration. These findings underscore the critical role of optimizing both cryoprotectant concentration and storage temperature to maintain high post-thaw viability and recovery in microalgal cultures, particularly in species sensitive to osmotic stress. Additionally, integrating gene expression analysis and omics approaches could provide valuable insights into the effects of preservation on the production of bioactive compounds such as pigments and antioxidants, with applications in aquaculture and biotechnology.

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Physiological, and Biochemical Responses of Shubunkin Goldfish, *Carassius auratus* (Linn.) Exposed to Inland Saline Groundwater

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***Abstract*:** Goldfish (*Carassius auratus*) are valued in ornamental aquaculture for their vibrant coloration and adaptability. This study examined their tolerance to inland saline water (ISW) and its effects on stress, growth, and survival. Over 120 days, goldfish were exposed to ISW, with an initial salinity of 12 ppt. Fish were conditioned and gradually acclimated to salinities of 0, 2, 4, 6, 8, and 10 ppt, increasing by 1 ppt every 2 hours. that salinity levels above 4ppt led to significant deviations in stress indicators, with marked changes in haematological, biochemical, and antioxidant responses at levels up to 10 ppt. Goldfish were able to adapt and grow in salinities up to 6 ppt, though levels below 4 ppt were optimal for health and performance. This research offers insights into goldfish adaptability in ISW, suggesting that salinities up to 6 ppt are manageable, with levels below 4 ppt most favourable, thereby supporting the potential of inland saline water in aquaculture.

***Keywords*:** *Carassius auratus*, Fish physiology, Growth, Goldfish, Inland saline water, Salinity

Salinization affects approximately 1,125 million hectares of global land, with about 76 million hectares impacted by human activities (Hossain 2019). This process occurs naturally but is often exacerbated by human factors, particularly in arid and semi-arid regions where practices such as excessive irrigation, waterlogging, and indiscriminate fertilizer use drive salt accumulation in soils (Verma et al., 2013). As agricultural productivity declines on these lands, they are increasingly being repurposed for inland saline aquaculture, providing an alternative use for degraded areas. Inland saline aquaculture leverages salt-affected regions to support fish farming, however, elevated salinity imposes physiological challenges for fish, impacting growth, survival, and metabolism. High salinity induces osmotic stress and ionic imbalances that can disrupt normal cellular processes, which are critical for growth and homeostasis (Khan 2019, Zhang et al., 2023). These stressors are compounded by the unique ionic profiles of inland saline water, which differ from seawater and require species to exhibit specific adaptive responses (Kim et al., 2017, Tian et al., 2020). Therefore, understanding which species can thrive in these environments is essential to optimize inland saline aquaculture practices. Freshwater species like goldfish, crucian carp, and molly have shown tolerance to saline conditions (Schofield et al., 2006, Singh et al., 2023). In Punjab, koi carp and Shubunkin goldfish have demonstrated adaptability to saline water, supporting potential for inland saline aquaculture in India (Bhatt 2018, Bhatt et al., 2018) and expanding inland aquaculture (Dhawan et al., 2010, Li et al., 2024). Goldfish (*Carassius auratus*) are highly adaptable to varying water conditions, making them ideal candidates for saline aquaculture. Their resilience offers a model for studying salinity's effects on growth and survival, with benefits for ornamental fish production and land rehabilitation. This study examines goldfish responses to different salinity levels in inland saline water to assess their viability in saline aquaculture.

Currently, no studies address the effects of inland saline water on the survival, growth, behavior, physiological responses, coloration, and stress responses of ornamental fish. Given the waterlogging and natural salinity issues in certain areas of North-Western India, it is essential to optimize rearing techniques for freshwater goldfish (*C. auratus* Linn.). Enhancing goldfish salinity tolerance could improve the socio-economic status of local farmers. Understanding how ISW's ionic composition differs from seawater and affects various fish species is also crucial. This study thus aims to assess the adaptability of goldfish to ISW by examining survival, growth, behavior, physiological responses, and coloration.

MATERIAL AND METHODS

Experimental environments: The study was conducted at the Instructional cum Research Farm, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India (30°90'45.1"N, 75°80'16.4"E) over a four-month period. Inland saline water, stock water: 15 ppt was obtained from the salt-affected and waterlogged region

of Shajrana, District Fazilka, Punjab (30°33'53.7"N, 74°11'92.2"E). To ensure water quality, the stock water was continuously aerated for 15 days and subsequently filtered through muslin cloth (20 µm). The filtered water was then diluted with freshwater (borewell water) to achieve salinity levels of 2, 4, 6, 8, and 10 ppt, with freshwater (0 ppt salinity) serving as the control. A total of one hundred eighty goldfish (*C. auratus var. shubhunkin*) were procured from a local market and kept in indoor cemented tanks for a one-month conditioning period. During this time, the fish were gradually acclimatized to the experimental salinities by increasing the salinity by 1 ppt every 2 hours until the desired salinity levels were reached. After acclimatization, the fish were stocked in glass aquaria (50 liters capacity) at a density of 10 fingerlings per aquarium. The experiment involved six treatments (three replicates for each treatment), with salinity levels ranging from 2, 4, 6, 8, and 10 ppt. All aquaria were continuously aerated with a diaphragm air blower (RS-15000, 220-240 V/50Hz, 20 Watt, 0.024 MPa). The experiment was conducted following a completely randomized design (CRD) to ensure proper randomization of treatments. The goldfish were provided commercial feed (OPTIMUM: 28% crude protein, 4% crude fiber, 3% crude fat, and 10% moisture) ad libitum twice a day, at 9–10 AM and 4-5 PM. Before each feeding, any remaining feed and faecal matter were siphoned out to maintain water quality. Water samples for physico-chemical analysis were collected daily between 09:00 and 10:00 AM. The parameters measured included temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), total hardness (TH), total alkalinity (TA), ammonia nitrogen (NH_3-N), and ionic composition (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-}), using standard methods outlined in APHA (2012).

Behavioral and survival observations: Fish survival and behavior were monitored daily across all treatments. The survival rate was determined using the following formula (Sultana et al., 2018):

Survival rate (%) = (Number of fish alive / Number of fish stocked) × 100.

Behavioral responses were categorized based on swimming activity (very active, less active, erratic swimming), feeding behavior (high, moderate, low appetite), and threat response (normal, modest, weak). The swimming behavior was assessed by observing the fish's movement within the water column, while feeding response was determined by the amount of uneaten feed remaining at the bottom of the tank (Lawson and Alake 2011). Stress-related behavioral reactions were noted periodically throughout the 120-day salinity exposure.

Sampling and analysis: At the end of the 120-day experimental period, the fish were fasted for 24 hours. Following this, they were anesthetized using 0.4 mL L^{-1} of 2-phenoxyethanol (1 mL L^{-1}) administered via 26G x 1/2" (0.45 × 13 mm) syringes (Hindustan Syringes & Medical Devices Ltd.). After the fish were anesthetized, they were weighed and measured before being returned to their respective tanks. Blood samples were obtained from the caudal veins of three fish per tank using heparinized syringes. The collected blood was centrifuged at 4000 g for 3 minutes at 4°C to separate the serum. The resulting serum was stored at -80°C for subsequent analysis (Bhatt et al., 2024).

Growth performance

Total body length and weight: These were measured monthly following standard procedures (Halver 1957).

Specific growth rate (SGR, % weight gain day^{-1})

SGR= log(final body weight (g)- log(initial body weight (g)/ t (time interval in days) x 100

Feed conversion ratio (FCR) = feed intake (g)/ weight gain (g)

Weight gain (%) = Final body weight (g) − Initial body weight (g)/Initial body weight (g) x 100

Blood metabolic profile

Reagents and materials: Total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin (Hb), hematocrit (HCT), total protein, albumin, and glucose (Cat. #120235 ERBA Mannheim Kit) were measured from fish serum according to the reference manual provided by ERBA Lachema s.r.o. A 50 µL serum sample was analyzed using a biochemical analyzer (CHEM-7, ERBA, Mannheim), following the standard protocol. Additionally, antioxidant parameters, including superoxide dismutase (SOD), lipid peroxidation (LPO), and glutathione reductase (GR), were assessed based on standard methodologies (Nicholls 1962, Nishikimi et al., 1972, Placer et al., 1966).

Statistical analysis: The data was analysed using SPSS 20.0 software (IBM SPSS Inc., USA) with Duncan's multiple-range test.

RESULTS AND DISCUSSION

Water quality parameters: Na^+ and Cl^- were the dominant ions, with higher concentrations than other cations and anions ($Na^+ > Mg^{2+} > Ca^{2+} > K^+$ and $Cl^- > SO_4^{2-}$). Significant differences were observed in water quality and ionic composition (Table 1). Except for temperature, all parameters and ion concentrations increased with salinity, with the highest values at 10 ppt and the lowest at 0 ppt (control). There were no significant differences in the physico-chemical parameters across the experimental tanks. All parameters remained within acceptable ranges for fish culture, consistent with Akinrotimi et al. (2007b). Parameters such as electrical conductivity (EC), total

alkalinity (TA), total hardness (TH), and the ionic composition ($Na^+ > Mg^{2+} > Ca^{2+} > K^+$ and $Cl^- > SO_4^{2-}$) exhibited trends parallel to the stock inland saline water (12 ppt). However, elevated ammonia nitrogen (NH_3-N) levels at higher salinities could represent a major stressor for freshwater fish (Bhatnagar and Singh 2010). There was significant increase in fish mortality with rising salinity, peaking at the highest salinity exposure.

Growth parameters: The survival rates were 100% at 0ppt and 2ppt, they began to decrease from day 45 in treatments with higher salinity (Table 2). At the completion of the experiment, survival rates varied between 80 and 100%. Growth metrics—such as mean final total body length (cm), body weight (g), total length gain (TLG), net weight gain (NWG), and specific growth rate (SGR) also showed significant variations), underscoring the detrimental effect of elevated salinity on both survival and growth (Table 2). Maintaining the salinity at or below 2 ppt is ideal for the extended rearing of goldfish, as growth performance significantly declined at salinity levels above 8ppt over the 120-day trial period. Küçük (2013) also demonstrated similar tolerances and growth trends under increased salinity. The combined influence of temperature (14.2 to 30.5°C range) and salinity notably impacted survival rates during prolonged exposure. The goldfish are typically hardy, their optimal performance at lower salinities (2 ppt) reinforces their

Table 1. Mean physico- chemical parameters of stock ISW and different salinity treatments

Parameters	ISW (Stock) 12 ppt	0 ppt	2 ppt	4 ppt	6 ppt	8 ppt	10 ppt
Temperature (^OC)	29.33	24.06^a	24.14^a	24.01^a	24.0^a	24.10^a	24.07^a
pH	8.87	7.41^d	7.99^c	8.28^b	8.30^b	8.46^a	8.48^a
DO (mg/L)	5.50	9.13^a	8.47^b	8.19^c	7.99^c	7.19^d	6.49^e
EC (mS/cm)	17.92	0.84^f	5.94^e	8.82^d	10.78^c	13.45^b	17.22^a
TA ($CaCO_3$ mg/L)	254.7	260^e	279^d	295^c	300^{bc}	305^b	317^a
TH ($CaCO_3$ mg/L)	2316.7	364.60^f	1045.60^e	1550.50^d	2168.80^c	2250.80^b	2699.90^a
NH_3-N (mg/L)	0.21	0.06^c	0.15^b	0.14^b	0.14^b	0.14^b	0.18^a
Ca^{2+} ($CaCO_3$ mg/L)	360.2	91.23^f	115.00^e	141.30^d	195.70^c	233.70^b	281.10^a
Mg^{2+} ($CaCO_3$ mgl^{-1})	587.3	83.29^f	231.50^e	333.20^d	466.20^c	558.10^b	586.60^a
Na^{2+} (mg/L)	765.9	19.80^f	192.90^e	264.20^d	364.30^c	460.40^b	592.60^a
K^+ (mg/L)	91.1	0.84^f	16.21^e	24.91^d	39.73^c	58.35^b	79.56^a
Cl^- (mg/L)	3539.7	72.50^f	621.20^e	950.70^d	1571.70^c	2194.50^b	2854.90^a
SO_4^{2-} (mg/L)	87.1	5.80^d	38.56^c	43.58^c	60.21^b	68.50^b	81.79^a

Values with same superscripts in a row do not differ significantly ($p \leq 0.05$).

Table 2. Survival and growth of shubunkin goldfish, *C. auratus* (L.) in different salinity treatments

Parameters	Days	0 ppt	2 ppt	4 ppt	6 ppt	8 ppt	10 ppt
Av. ITBL (cm)	0	8.04^a	8.10^a	8.15^a	8.19^a	8.26^a	8.25^a
Av. FTBL (cm)	120	8.11^{ab}	8.19^{ab}	8.17^{ab}	8.23^a	8.01^{ab}	7.76^b
Av. IBW (g)	0	6.40^a	6.40^a	6.80^a	6.60^a	6.50^a	6.30^a
Av. FBW (g)	120	7.98^a	7.28^b	7.38^b	7.01^b	5.37^c	5.16^d
Survival (%)	0-30	100.0^a	100.0^a	100.0^a	100.0^a	100.0^a	100.0^a
	30-60	100.0^a	100.0^a	86.66^a	86.66^a	86.66^a	86.66^a
	60-90	100.0^a	100.0^a	86.66^a	86.66^a	80.00^b	60.00^b
	90-120	100.0^a	93.33.0^a	80.00^{ab}	80.00^{ab}	80.00^{ab}	60.00^b
TLG		0.07^a	0.09^a	0.02^{ab}	0.04^{ab}	-0.25^c	-0.48^c
NWG		1.58^a	0.89^{ab}	0.58^b	0.41^b	-1.14^c	-1.12^c
SGR		1.32^a	0.73^{ab}	0.48^b	0.34^b	-0.95^c	-0.93^c

Values with same superscripts in a row do not differ significantly ($p \leq 0.05$).
Av.= Average; ITBL= Initial total body length; FTBL= Final total body length,
IBW= Initial body weight, FBW= Final body weight, TLG = Total length gain; NWG= Net weight gain,
SGR = Specific growth rate

categorization as stenohaline freshwater fish (Luz et al., 2008). The reduction in growth at elevated salinities can likely be attributed to physiological and metabolic disruptions triggered by salinity stress (Mangat and Hundal 2014). Although goldfish could survive at a salinity of 8 ppt, their growth was compromised, likely due to a reduction in appetite and decreased feed conversion efficiency at these higher salinity levels (Luz et al., 2008).

Fish behavior was monitored through assessments of swimming activity, feeding responses, and morphological characteristics, including skin coloration, body fragility, and mucus secretion. Deviations from typical behavior, such as reduced activity, lethargy, diminished feeding responses, skin discoloration, increased fragility, and excessive mucus production, were observed at a salinity level of 6 ppt after 45 days (Table 3).

Blood Metabolic Profile

Haematological parameters: TEC, TLC and Hct showed a progressive increase with rising salinity levels from 0 ppt to 10ppt. Conversely, hemoglobin (Hb) concentrations were highest at 0 ppt and significantly decreased to their lowest at 4 ppt (Table 4). TEC, TLC, and Hct levels were observed to increase progressively with rising salinity from 0 to 10 ppt. Extended exposure to lower temperatures combined with elevated salinity brought about significant shifts in these physiological parameters, suggesting that stress induced by these conditions required compensatory adjustments to maintain homeostasis (Hosseini et al., 2011). Under salinity stress, heightened Hct levels often indicate water loss resulting from ionic imbalances between the fish's internal environment and the external conditions. Initially, during saltwater acclimation, Hct levels may increase but typically stabilize to pre-exposure values over time. Stress conditions can also elevate catecholamine release, which stimulates TEC production as a response to reduced oxygen levels, potentially resulting in increased TEC and Hct measurements. Typically, higher TEC correlate with increased Hb levels, essential for respiration. However, TEC rose with salinity, Hb levels declined, suggesting poor cell quality or reduced hemoglobin content per cell. This discrepancy likely indicates an adaptive response, with abnormal TEC formation possibly leading to anemia under salinity stress. TLC also increased at higher salinities, likely reflecting an immune response mediated by elevated cortisol (Gomes et al., 2003). These findings reveal adaptive shifts in respiratory and immune functions, underscoring the need for further study into physiological responses to salinity in fish.

Biochemical and antioxidant parameters: There was results reveal a significant decrease in serum blood glucose and total protein levels up to 8 ppt salinity, followed by a

Table 3. Behavioural and morphological responses in shubunkin goldfish, *C. auratus* (L.) in different salinity treatments during the experimental period

Behaviour	Days	0 ppt	2 ppt	4 ppt	6 ppt	8 ppt	10 ppt
Swimming activity	0-15	A	A	A	A	A	VA
	30	A	A	A	A	LA	LA
	45-75	A	A	LA	LA	LA	S
	90-120	A	A	LA	LA	S	S
Feeding response	0-45	HAp	HAp	Hap	HAp	Hap	Hap
	60-75	HAp	HAp	Hap	HAp	Hap	Lap
	90-105	HAp	HAp	Hap	HAp	Lap	Lap
	120	HAp	HAp	Lap	LAp	Lap	Lap
Colouration	0-30	NC	NC	NC	NC	NC	NC
	45	NC	NC	NC	NC	DC	DC
	60-120	NC	NC	NC	DC	DC	DC
Body fragility	0-30	NF	NF	NF	NF	NF	NF
	45-60	NF	NF	NF	HF	HF	HF
	75-120	NF	NF	HF	HF	HF	HF
Mucus	0-30	NM	NM	NM	NM	NM	NM
	45-120	NM	NM	NM	EM	EM	EM

Swimming Activity - A = Active, LA = Less Active, S = Sluggish
Feeding Response – HAp – High Appetite, LAp = Low Appetite
Colouration – NC = Normal colouration, DC = Dull colouration
Body fragility- NF = Normal fragility, HF = High fragility
Mucus- NM = Normal Mucus, EM = Enhanced Mucus

marked increase at 10 ppt (Table 4). Albumin and globulin levels displayed significant modulation across salinity levels, with maximum values at 10 ppt (Table 4). Correspondingly, the Alb/Glb ratio decreased up to 4 ppt, increased at 6 ppt, and then significantly declined at 8 ppt and 10 ppt (Table 4). Salinity stress may trigger glucogenolysis to meet energy demands, leading to elevated blood glucose and reduced liver glycogen. Huang et al. (2006) reported that increased salinity raises energy consumption, initially met through glucose and lipid metabolism, with proteins utilized when glucose is insufficient. Total serum protein decreased up to 8 ppt salinity, possibly due to altered amino acid metabolism and protein breakdown, as observed in common carp and rohu under transport stress. Albumin and globulin levels followed a similar trend, decreasing up to 4 ppt and increasing thereafter, indicating immune system activation. Elevated protein levels and reduced Alb/Glb ratios at higher salinity suggest enhanced defense mechanisms. The results highlight the adaptive capacity of goldfish at salinities of 2–4 ppt, where homeostasis is maintained, also reflected in optimal growth.

Among antioxidant parameters, superoxide dismutase (SOD) reached its highest levels at 6 ppt, while lipid peroxidation (LPO) and glutathione reductase (GR) peaked at 6 and 10 ppt, respectively. LPO and GR levels showed a significant increase (across all salinity treatments (Table 4). The activities of antioxidant enzymes (SOD, LPO, and GR) increased noticeably at higher salinity levels, indicating oxidative stress. This suggests that an imbalance between reactive oxygen species production and the antioxidant defenses of the fish occurs under these stressful conditions. These findings are consistent with prior studies (Lushchak and Bagnyukova 2006, Birnie-Gauvin 2017) which also reported elevated antioxidant enzyme levels as a response to environmental stressors, underscoring the importance of antioxidant mechanisms in protecting cells from oxidative damage.

Carotenoid analysis of fish skin and muscle: Carotenoid content demonstrated a significant reduction at salinity levels above 4 ppt, accompanied by a visible bleaching of yellow pigmentation in the skin. The increase in carotenoid levels was observed at 8 ppt, although no significant differences were detected between the 0 and 2 ppt treatments. At the higher salinity range (8–10 ppt), both skin brightness and carotenoid content declined further, intensifying the bleaching effect (Table 4).

Goldfish were able to preserve their normal coloration and carotenoid levels up to 2 ppt salinity, indicating that they could maintain their regular metabolic and physiological processes at lower salinity concentrations. However, as salinity increased beyond this threshold, metabolic

Table 4. Haematological, biochemical, antioxidant parameters and carotenoid content of shubunkin goldfish, *C. auratus* (L.) in different salinity levels at the completion of the experiment

Parameters	0 ppt	2 ppt	4 ppt	6 ppt	8 ppt	10 ppt
Haematological parameter						
Hb (g %)	5.83^{a}	4.50^{b}	3.50^{b}	3.56^{b}	3.53^{b}	3.60^{b}
Hct (%)	5.96^{d}	7.23^{c}	7.90^{b}	8.26^{b}	8.33^{b}	9.03^{a}
TEC ($x10^{6}$ mm^{3-1})	1.31^{e}	2.10^{d}	2.90^{c}	3.56^{b}	3.86^{ab}	4.46^{a}
TLC ($x10^{3}$ mm^{3-1})	3.32^{c}	3.40^{c}	4.04^{bc}	4.83^{b}	6.19^{a}	6.28^{a}
Biochemical parameters						
Glucose (g dl^{-1})	98.03^{b}	44.63^{d}	41.04^{f}	43.40^{e}	61.50^{c}	191.2^{a}
Protein (g dl^{-1})	4.24^{b}	3.75^{c}	1.97^{e}	2.85^{d}	3.74^{c}	9.10^{a}
Albumin (g dl^{-1})	1.88^{a}	1.09^{b}	0.45^{c}	1.12^{b}	1.15^{b}	1.13^{b}
Globulin (g dl^{-1})	2.35^{c}	2.65^{b}	1.52^{d}	1.73^{d}	2.59^{b}	7.97^{a}
Alb/Glb ratio (g dl^{-1})	0.79^{a}	0.41^{b}	0.29^{bc}	0.74^{a}	0.44^{b}	0.14^{c}
Antioxidant parameters						
SOD (U $mgHb^{-1}$)	0.15^{b}	0.20^{b}	0.22^{b}	0.50^{a}	0.40^{a}	0.47^{a}
LPO (nmol MDA G Hb^{-1})	0.10^{d}	0.14^{d}	0.40^{d}	2.07^{c}	3.22^{b}	4.43^{a}
GR (Mm l^{-1})	2.01^{e}	2.49^{d}	3.03^{c}	3.41^{b}	3.92^{a}	4.29^{a}
Carotenoid content						
Carotenoid (µg g^{-1}) 35. 74 (At initiation-0 day)	49.33	37.15	36.9	36.45	38.41	35.58

Values with same subscripts in a row do not differ significantly ($p \leq 0.05$)
TEC= Total erythrocyte count, TLC= Total leucocyte count, Hb= Haemoglobin, Hct= Haematocrit value, SOD= Superoxide dismutase, LPO= Lipid peroxidation and GR= Glutathione reductase

disruptions occurred, leading to a breakdown in physiological functions. These changes likely contributed to the observed decline in skin pigmentation, as well as reduced growth and survival. Lawson and Alake (2011), also observed that goldfish exposed to varying salinity levels experienced alterations in their coloration and overall health. The salinity-induced stress appears to have impaired pigmentation, which may have been a consequence of compromised physiological functions under higher salinity conditions.

CONCLUSION

This study highlights the adaptability of *Carassius auratus* to inland saline water, demonstrating that goldfish can grow and survive in salinities up to 6 ppt. However, optimal performance based on survival, growth, haematological, biochemical, antioxidant responses, and coloration was observed at salinity levels ≤4 ppt. These results suggest that maintaining salinity levels at or below 4 ppt is ideal for the long-term rearing of freshwater ornamental goldfish in inland saline water. the experiment was conducted under controlled laboratory conditions, further field-based studies are necessary to evaluate the effects of salinity in more dynamic, natural environments, accounting for additional ecological factors.

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Comparative Assessment of Anuran Diversity and Morphometric Ratios from Aquatic Ecosystem of Sub Mountainous and Plain Zones of Punjab

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Abstract: Present study investigates four anuran species *Duttaphrynus melanostictus*, *D. stomaticus*, *Euphlyctis cyanophlyctis* and *Hoplobatrachus tigerinus* belonging to families Bufonidae and Dicroglossidae under order anura in aquatic ecosystem (village ponds) of plain zone (Ludhiana) and sub mountainous zone (SBS Nagar) of Punjab (India) during 2022 and 2023. Higher anuran population (2.99 to 5.15 individuals/month/transect) was in sub mountainous zone followed by plain zone (2.02 to 3.98 individuals/month/transect). Seasonal activity of anurans was from May to December and during whole year with higher population during July and August month. A 10.27% increase in population was in sub mountainous zone during 2023 as compared to previous year whereas was only 8.87% in plain zone. Inside pond, *H. tigerinus* showed a significant 46.15-72.22% variation in population in sub mountainous zone as compared to plain zone which was significantly different from other anuran species. However, in outside (periphery) of village ponds, *E. cyanophlyctis* showed highest (47.56-47.61%) population variation in sub mountainous zone as compared to plain zone which was significantly different from other anuran species. Biodiversity indices, like Shannon-Wiener index and Simpson's index improved in 2023 as compared to previous year in plain and sub mountainous zone, respectively. Morphometric analysis revealed significant sexual dimorphism, particularly in *H. tigerinus*, with interspecific variations reflecting adaptations to different microhabitats. Significant seasonal variations and increase in anuran population was in sub mountainous zone consistently supported higher densities than plain zone. Enhanced biodiversity indices and positive correlations with weather parameters indicate improved ecosystem health over time. Morphometric differences, particularly in *H. tigerinus*, highlight species-specific adaptations to environmental niches, advancing our understanding of anuran ecological roles.

Keywords: Anuran, Biodiversity indices, Diversity, Frog, Morphometric ratios, Toad

Anurans (frogs and toads) are one of the most diverse and widespread groups of amphibians, playing vital roles in both terrestrial and aquatic ecosystems. With approximately 7,500 known species worldwide, representing about 88% of all amphibian species (Frost 2023). Their diversity spans continents, with concentrations in tropical regions, particularly Brazil, Colombia and the Indo-Malayan and Afrotropical realms (Hof et al., 2011). In India, approximately 414 anuran species have been recorded, with the Western Ghats being a significant hotspot, harbouring about 159 endemic species (Biju and Bossuyt 2009). However, around 41% of amphibian species, including anurans, are threatened (IUCN 2024), primarily due to habitat loss, climate change, diseases like chytridiomycosis and invasive species. Village ponds are crucial habitats for anurans, providing breeding sites, foraging areas and population connectivity in agricultural landscapes. These ponds are influenced by factors such as water depth, shoreline complexity, vegetation and seasonal variations, which shape anuran diversity and abundance (Jadhav et al., 2023). Larger ponds with richer vegetation typically support more species, while environmental changes and human activities increasingly affect these communities (Kumar et al., 2024). Conservation efforts must integrate local habitat protection and landscape-level approaches, such as maintaining pond networks, protecting shoreline vegetation and managing water quality (Patel et al., 2023). The use of modern technologies like environmental DNA sampling, automated acoustic monitoring and remote sensing is improving understanding of anuran population dynamics and habitat use (Rana et al., 2023, Sharma et al., 2024;).

Present research was carried out with an objective of comparing the status of anuran diversity in village ponds of sub mountainous and plain zones of Punjab state. By examining these ecosystems, the study seeks to understand how environmental factors influence anuran communities in different ecological settings (Sharma et al., 2024). Future research priorities should focus on long-term monitoring of population trends, assessment of emerging threats and development of effective conservation strategies that consider both ecological requirements and human needs in these important ecosystems.

MATERIAL AND METHODS

Study was carried out during 2022 and 2023 at selected villages (five each) of district Ludhiana and district Shaheed Bhagat Singh (SBS) Nagar which belongs to plain zone and sub mountainous zone, respectively. Ludhiana is located

between the coordinates 30.9010°N,75.8573°E, in the central region of Punjab State, while SBS Nagar lies between the coordinates 31.0913°N, 76.0392°E. The five selected aquatic ecosystems (village ponds) of district Ludhiana included Bhattian, Chak Kalan, Alamgir, Kaind and Jassowal whereas of district SBS Nagar includes Langroya, Amargarh, Ghamour, Rurki Kalan and Hussain Chak.

Sampling of anuran diversity: To study diversity and abundance of anurans, Visual Encounter Survey Method (Heyer 1994) was used for estimating the anuran population in a belt transect of 50×4 m along a survey path at fortnight interval and was pooled at month level from May to December during 2022 and 2023. Each anuran species was captured with hand wearing gloves from selected locations, photographed for identification, checked for any morphological abnormalities and released back in their natural habitat. Anurans were identified from ZSI (Zoological Survey of India), Pune and using keys published by Daniel (2005). Data on atmospheric temperature (^{0}C), relative humidity (%) and rainfall (mm) were taken from Department of Climate Change and Agricultural Meteorology, PAU, Ludhiana and Ballowal Sounkri for Ludhiana and SBS Nagar, respectively.

Calculation of various indices

Simpson index

$$D = \frac{\sum_{i=1}^{S} ni(ni-1)}{N(N-1)}$$

Where, D = Simpson index

n_i = Total number of individuals in a particular species

N = Total population of all species

Shannon-Wiener index

$$H' = -\Sigma (pi \times \ln pi)$$

Where,

H' = Shannon-Wiener index

p_i = Total number of individuals in a particular sample

$$J = \frac{H'}{\ln(S)}$$

Pielou's species evenness

Where,

J = Species evenness

H'= Shannon-Wiener index

S= is the number of species in the community.

Statistical analysis: For diversity of anurans, the data was analysed with Tukey's test with p value = 0.05 using SPSS software. Different indices like Shannon-Weiner diversity index, species evenness, species richness and Simpson's index were calculated using different formulas and PAST (paleontological statistics) software. Correlation coefficient was calculated to find relation between anuran diversity and atmospheric temperature (^{0}C), rainfall (mm) and relative humidity (%).

RESULTS AND DISCUSSION

The present study provides significant insights into anuran community dynamics in the aquatic ecosystems (village ponds) of the plain zone (district Ludhiana) and sub-mountainous zone (SBS Nagar)**.** Four anuran species (*Duttaphrynus melanostictus, D. stomaticus, Euphlyctis cyanophlyctis,* and *Hoplobatrachus tigerinus*) from the families Bufonidae and Dicroglossidae were documented in the village ponds of both zones. These species demonstrated seasonal activity patterns from May to December, with peak abundance during July and August, which coincides with the rainy season in Punjab. This phenological pattern aligns with observations from similar tropical and subtropical regions worldwide (Blaustein et al., 2012, Todd et al., 2011) suggesting consistent breeding strategies across geographical ranges in response to monsoon-dependent ecosystems.

Population in aquatic ecosystem of plain zone and sub mountainous zone: In plain zone (district Ludhiana), from aquatic ecosystem (village ponds) of selected villages, mean anuran population was 2.93 to 3.19 during 2022 and 2023, respectively, being highest in village Jassowal (and lowest in Alamgir) (Table 2).The maximum variation in population

Table 1. Inventory of anuran species from aquatic ecosystem (village ponds) of plain zone (district Ludhiana) and sub mountainous zone (SBS Nagar)

Common name	Scientific name	Family	Order	Ludhiana	SBS Nagar
Common Asian toad	*Duttaphrynus melanostictus* (Schneider 1799)	Bufonidae	Anura	#	#
Indian marble toad	*Duttaphrynus stomaticus* (Lütken 1864)			#	#
Indian skittering frog	*Euphlyctis cyanophlyctis* (Schneider 1799)	Dicroglossidae		#	#
Indian bull frog	*Hoplobatrachus tigerinus* (Daudin 1802)			#	#

* (#) Indicates presence of species

during 2023 was in Bhattian with increase of 30.55% followed by Kaind (18.40%), while decrease in population was recorded in Alamgir (9.82%) as compared to 2022. The overall increase of 8.87% anuran population was in plain zone (district Ludhiana) during 2023 as compared to 2022 (Table 2). Similarly, in sub mountainous zone (district SBS Nagar), from aquatic ecosystem (village ponds) of selected villages, anuran mean population was 3.70 to 4.08 during 2022 and 2023, respectively, being highest in Hussain Chak and lowest in Amargarh (Table 2).The maximum variation in population during 2023 was in Rurki Kalan with increase of 16.53% followed by Amargarh (10.36%), Hussain Chak (9.80%), Ghamour (8.99%) and Langroya (4.95%) as compared to 2022. overall increase of 10.27% anuran population was in sub mountainous zone(district SBS Nagar) during 2023 as compared to 2022.

Comparison of anuran population in aquatic ecosystem of plain zone and sub mountainous zone: Higher mean anuran population was in village ponds of district SBS Nagar (sub mountainous zone) as compared to district Ludhiana (plain zone). During 2022 and 2023, among outside (periphery) of all the selected aquatic ecosystems (village ponds), *E. cyanophlyctis* showed highest (47.56-47.61%) variation in population at SBS Nagar as compared to district Ludhiana which was significantly different from other anuran species. *D. stomaticus* and *D. melanostictus* showed 28.57-35.91% and 19.64-22.12% variation in population at SBS Nagar as compared to district Ludhiana and was statistically at par with each other. However, *H. tigerinus* showed only 1.47-14.10% variation in population at SBS Nagar as compared to district Ludhiana. Population trend of anurans inside the aquatic ecosystems were more pronounced. *H. tigerinus* showed significant 72.22% variation in population at SBS Nagar as compared to district Ludhiana. However, in 2023, *H. tigerinus* showed a significant 46.15% variation in population at SBS Nagar as compared to district Ludhiana which was significantly different from *E. cyanophlyctis* (7.48%) (Fig. 1, 2). The observed population dynamics revealed spatial and temporal variations between the two selected zones, with the sub-mountainous zone showing a higher population increase (10.27%) compared to the plain zone (8.87%) from 2022 to 2023. This difference in population can primarily be attributed to variations in environmental parameters, particularly rainfall (1396 mm and 1032 mm in sub-mountainous and plain zones, respectively) and relative humidity (0.83% higher in the sub-mountainous zone than in the plain zone). These findings correspond with global studies highlighting the crucial role of precipitation patterns in amphibian population dynamics (Grant et al., 2016, Hopkins, 2007).

Diversity indices in aquatic ecosystem of plain zone and sub mountainous zone: In aquatic ecosystem (village ponds) of district Ludhiana, the Shannon-Wiener index, Pielou's index and Simpson's index increased from 0.901, 0.567 and 0.335 to 0.924, 0.884 and 0.349, respectively in 2023 as compared to 2022 (Table 4). Likewise, in aquatic ecosystem (village ponds) of district SBS Nagar, the

Table 2. Anuran population in aquatic ecosystem (village ponds) of plain zone (district Ludhiana) and sub mountainous zone (SBS Nagar) during 2022 and 2023

Villages	Mean population (2022)	Mean population (2023)	Per cent population variation in 2023 over 2022
Plain zone (district Ludhiana)			
Chak Kalan	3.14[a]	3.27[a]	+4.14
Bhattian	2.52[a]	3.29[b]	+30.55
Kaind	2.88[a]	3.41[b]	+18.4
Alamgir	2.24[a]	2.02[a]	-9.82
Jassowal	3.87[a]	3.98[a]	+2.84
Total	2.93[a]	3.19[b]	+8.87
Sub mountainous (SBS Nagar)			
Hussain Chak	4.69[a]	5.15[a]	+9.80
Rurki Kalan	3.75[a]	4.37[b]	+16.53
Ghamour	3.67[a]	4.00[a]	+8.99
Langroya	3.43[a]	3.60[a]	+4.95
Amargarh	2.99[a]	3.30[a]	+10.36
Total	3.70[a]	4.08[b]	+10.27

*Statistical analysis (showed significant result with p value = 0.05
*Mean value followed by same letter (a,b) in the given table above are not significantly different as per t -Test

Table 3. Diversity indices for anuran population in selected aquatic ecosystems (village ponds) of plain zone (district Ludhiana)and sub mountainous zone (SBS Nagar)

Indices	Aquatic ecosystem	
	2022	2023
Ludhiana		
Shannon- Wiener's index	0.901	0.924
Pielou's evenness index	0.567	0.884
Simpson's index	0.335	0.349
Species richness	4.00	4.00
SBS nagar		
Shannon- Wiener's 3 index	0.932	0.951
Pielou's evenness index	0.892	0.906
Simpson's index	0.780	0.799
Species richness	4.00	4.00

Shannon-Wiener index, Pielou's index and Simpson's index increased from 0.932, 0.892 and 0.780 to 0.951, 0.906 and 0.799, respectively in 2023 as compared to 2022. However, species richness (four) remained constant during 2022 and 2023 for both the zones under study.

Correlation between anuran population and weather parameters: Strong positive correlations were observed between anuran populations and mean atmospheric temperature and relative humidity in aquatic ecosystem (village ponds) of district Ludhiana and SBS Nagar (Fig. 3 to 6). The stronger correlation between environmental parameters and anuran abundance in 2023 (temperature: r=+0.394 to 0.533; relative humidity: r=0.536 to 0.820) suggests increasing environmental dependency, which possibly reflects adaptive responses to changing climatic conditions, a phenomenon also noted in other regions (Carey and Alexander 2003, Walls et al., 2013). The improvement in biodiversity indices from 2022 to 2023 (Shannon-Wiener index: 0.901 to 0.924 and 0.932 to 0.951 in Ludhiana and SBS Nagar, respectively) indicates enhanced community stability and evenness, despite constant species richness. This pattern mirrors findings from long-term studies in other agricultural landscapes (Cushman 2006, Khatiwada et al., 2016), suggesting that well-maintained village ponds can serve as effective refugia for anuran populations. The observed stability in species composition, coupled with increasing diversity indices, may indicate successful habitat utilization strategies and resource partitioning among species, as documented in similar agroecosystems globally (Ramesh et al., 2013, Dodd 2010). The stronger correlations between environmental parameters and population dynamics in 2023 raise important considerations about climate change impacts on amphibian communities. This increasing environmental dependency aligns with global observations of amphibian responses to climate change (Urban et al., 2016, Pounds et al., 2006), suggesting potential vulnerability to future climatic variations. The maintenance of stable populations despite these pressures indicates some level of resilience in these agricultural wetland systems, possibly due to the availability of suitable microhabitats and breeding sites (Semlitsch, 2008). However, several factors warrant careful consideration for long-term conservation. First, the constant species richness despite improving diversity indices suggests a possible ceiling effect in these modified landscapes, highlighting the need for habitat enhancement strategies (Gardner et al., 2007). Second, the strong environmental correlations indicate potential vulnerability to climate change, necessitating adaptive management approaches (Shoo et al., 2011). Third, the pronounced sexual dimorphism and morphological variations suggest complex ecological relationships that need preservation through habitat conservation (Stuart et al., 2004).

Morphometric ratios of male and female anuran species: Morphometric analysis of four anuran species revealed variations in body proportions across species and between males and females. The overall mean morphometric variation across all parameters and in significant differences observed in key ratios. The head length to snout-vent length (HL: SVL) ratio exhibited sexual dimorphism, particularly in

Table 4. Per cent increase in morphometric parameters (pooled mean of 2022 and 2023) of anuran species in aquatic ecosystem (village ponds) of sub mountainous zone (SBS Nagar) over plain zone (Ludhiana)

Morphometric parameters	*D. stomaticus*		*D. melanosticus*		*E. cyanophlyctis*		*H. tigerinus*		Mean
	Male	Female	Male	Female	Male	Female	Male	Female	
HL: SVL	7.41	14.71	7.14	12.9	3.85	17.14	34.45	20.13	14.72
HL: HW	9.23	10.00	7.46	2.74	11.59	11.27	5.56	34.57	11.55
HL: HD	10.28	6.96	6.25	1.68	7.02	4.96	5.47	0.71	5.42
SL: HL	4.76	4.55	5.13	8.89	12.82	8.16	7.02	11.29	7.83
SL: SVL	7.14	5.88	10.53	9.52	7.14	6.25	12.00	18.75	9.65
EN: NS	3.25	3.76	1.55	5.47	6.96	3.85	3.64	30.17	7.33
EN: HL	5.26	3.85	5.88	26.09	11.76	19.05	21.43	14.29	13.45
ED: HL	9.76	2.08	12.9	14.29	5.88	8.89	22.22	12.00	11.00
ED:SL	32.79	15.38	2.50	1.16	1.12	33.78	5.00	24.49	14.53
ED: SVL	9.09	6.25	8.33	35.71	27.78	24.00	20.00	75.00	25.77
ED: EN	6.2	2.01	0	1.61	4.26	0	5.83	4.20	3.01
Mean	9.56	6.86	6.15	10.91	9.11	12.49	12.97	22.33	11.3

H. tigerinus (males: 34.45%, females: 20.13%) and *E. cyanophlyctis* females (17.14%), with a mean ratio of 14.72% across species. Head proportions, measured through HL: HW ratio, showed high variation (11.55%), with *H. tigerinus* females displaying the higher value (34.57%) as compared to *D. melanosticus* females (2.74%). The head length to head

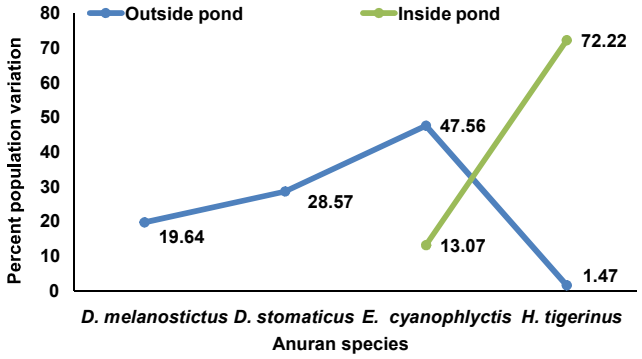

Fig. 1. Comparison of anuran population in aquatic ecosystem (village ponds) of plain zone (district Ludhiana) and sub mountainous zone (SBS Nagar) during 2022

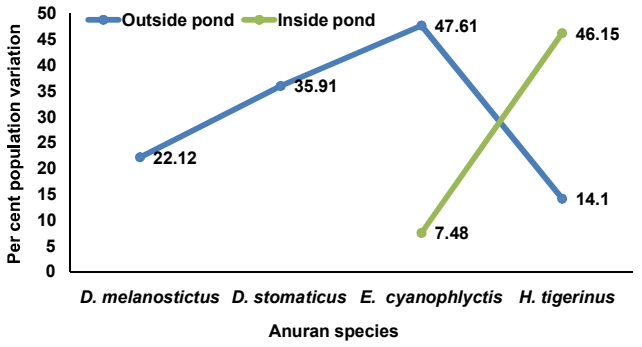

Fig. 2. Comparison of anuran population in aquatic ecosystem (village ponds) of plain zone (district Ludhiana) and sub mountainous zone (SBS Nagar) during 2023

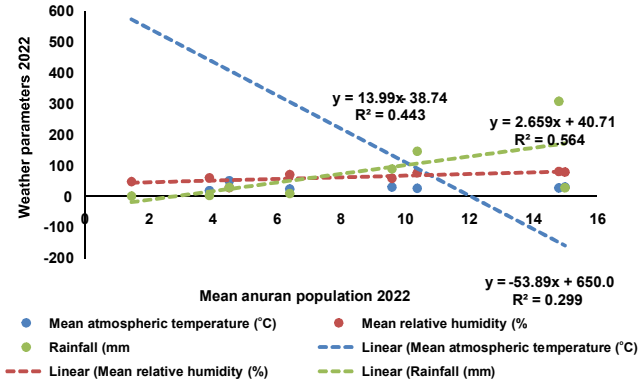

Fig. 5. Correlation between mean anuran population and weather parameters in aquatic ecosystem (village ponds) of sub mountainous zone (SBS Nagar) during 2022

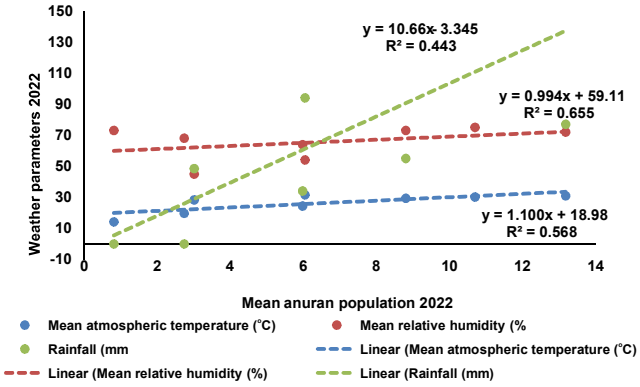

Fig. 3. Correlation between mean anuran population and weather parameters in aquatic ecosystem (village ponds) of plain zone (district Ludhiana) during 2022

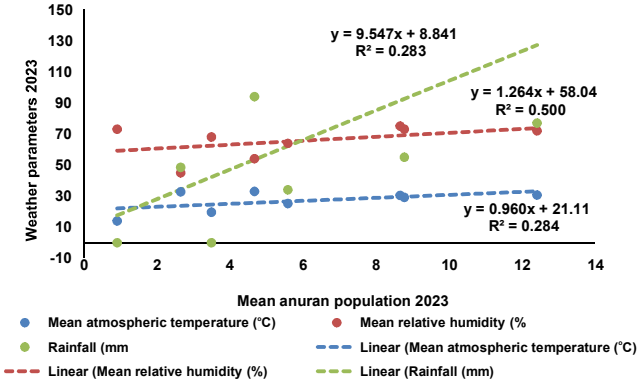

Fig. 4. Correlation between mean anuran population and weather parameters in aquatic ecosystem (village ponds) of plain zone (district Ludhiana) during 2023

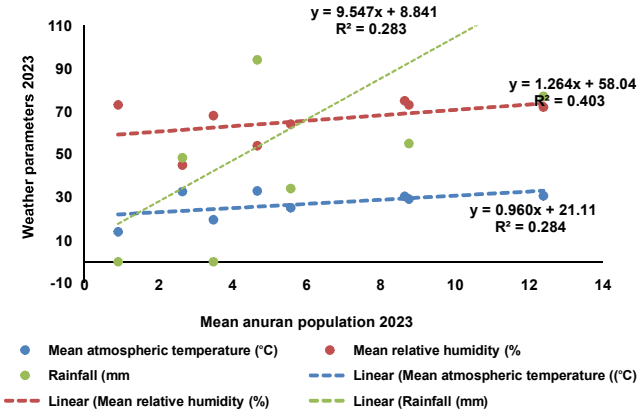

Fig. 6. Correlation between mean anuran population and weather parameters in aquatic ecosystem (village ponds) of sub mountainous zone (SBS Nagar) during 2023

depth ratio (HL: HD) ranged from 10.28% in *D. stomaticus* males to 0.71% in *H. tigerinus* females. SL:HL and SL:SVL ratios increased between 7.83% and 9.65% respectively. For ED: SVL ratios (mean 25.77%), *H. tigerinus* females exhibited higher value (75.0%). Species-specific analysis revealed *H. tigerinus* as the most morphometrically variable species (male: 12.97%, female: 22.33%), followed by *E. cyanophlyctis* (male: 9.11%, female: 12.49%), while *D. melanosticus* (male: 6.15%, female: 10.91%) and *D. stomaticus* (male: 9.56%, female: 6.86%) showed low variations. Sexual dimorphism was most pronounced in *H. tigerinus*, particularly in HL: SVL, HL:HW and ED:SVL ratios (Table 4). The observed interspecific and intersexual variations in morphometric parameters suggest species-specific adaptations to different microhabitats and ecological niches within the study area. These morphological differences likely reflect evolutionary adaptations to different microhabitat utilization and reproductive strategies, consistent with patterns observed in other anuran communities worldwide (Wells 2007). The variation in head and body proportions across species (mean morphometric variation: 11.3%) suggests niche differentiation and resource partitioning strategies, similar to findings reported in other tropical anuran assemblages (Richter-Boix et al., 2007, Vitt and Caldwell 2014;). These findings have important implications for amphibian conservation in village ponds globally. The successful maintenance of anuran populations in village ponds demonstrates the potential of these anthropogenic habitats as conservation units, provided they are properly managed (Hazell et al., 2004). However, continued monitoring is essential to understand long-term population trends and their responses to environmental changes (Egea-Serrano et al., 2012), particularly in the context of global climate change and agricultural intensification.

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Exploratory Study of Decline of Lavender Cultivation in Chamba District of Himachal Pradesh: Economic Analysis

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Abstract: This study examined the economic viability of lavender cultivation in Chamba, identifying key challenges confronted by farmers to develop a comprehensive framework for promoting lavender farming. The total cost of establishment of lavender crop was Rs.2.63 lakhs per hectare and total cost of cultivation of lavender from second year onwards was Rs.1.59 lakhs per hectare per year. The net present value of lavender cultivation at 8, 12 and 15 per cent of discount rates was Rs.9.05 lakh, Rs.7.04 lakh and Rs.5.86 lakh, respectively. The BC ratio at discount rate of 8, 12 and 15 per cent was 1.79, 1.73 and 1.68 respectively, which was more than unity indicating the worthiness of investment on lavender cultivation. The IRR for lavender cultivation was 67 per cent. The lavender farmers encountered significant challenges, primarily stemming from poor marketing facilities, high wages of labour and lack of government support. The research recommends developing farmer-centric marketing systems and promoting supportive government initiatives for lavender farmers, encompassing subsidies, training and market connectivity.

Keywords: Net present value (NPV), Internal rate of returns (IRR), Benefit-cost ratio

Lavender (*Lavandula* spp.) is a genus of flowering plants in the mint family, Lamiaceae, known for its fragrant purple flowers and aromatic foliage. Native to the Mediterranean region, lavender thrives in well-drained soils and prefers sunny, dry environment. Cultivated primarily for its essential oils, culinary uses, and ornamental value, lavender offers lucrative opportunities for farmers and entrepreneurs. Its low water and nutrient requirements make it an attractive option for sustainable agriculture. The essential oil extracted from lavender has a wide range of uses, including perfumes, cosmetics and medicinal purposes (Kumar and Verma 2023). The true lavender oil comes from *L. officinalis syn. angustifolia* which is the most highly priced among all the lavender oils. The global production of this oil is approximately 200 tonnes annually.

Lavender was introduced to India by British colonizers, who bought it from Europe for ornamental and medicinal purposes. Post-independence, Indian research institutions, notably, the Indian Institute of Horticultural Research (IIHR), facilitated lavender's cultivation expansion through targeted agronomic interventions, resulting in its establishment in regions with favorable climatic conditions, such as the Nilgiri Hills, Western Ghats and Himalayan foothills. Presently India produces approximately 100-150 tonnes of lavender oil annually, with key cultivation regions including Tamil Nadu, Kerala, Himachal Pradesh, Uttarakhand and Jammu & Kashmir.

Chamba, a picturesque district in Himachal Pradesh, India, is known for its favorable climate and soil conditions, making it an ideal location for lavender cultivation. The Institute of Himalayan Bioresource Technology (IHBT) successfully introduced lavender cultivation in Chamba district, in 2000 and trained over 500 farmers on cultivation, processing and marketing. The introduction of lavender had generated additional income for farmers, created employment opportunities, and contributed to soil and biodiversity conservation. After experiencing a successful introduction and growth phase (2000 to 2010), lavender cultivation in Chamba district experienced a significant decline between year 2010 and 2015. Despite its potential for high returns and employment generation, this decline has raised concerns among policymakers, farmers and researchers, highlighting the need for an in-depth analysis of the economic factors contributing to this trend.

To revitalize this industry, it is essential to assess the economic viability and financial feasibility of lavender cultivation. The present study aims to conduct an in-depth economic analysis of lavender cultivation in Chamba district of Himachal Pradesh, identifying the challenges faced by famers and exploring potential solutions.

MATERIAL AND METHODS

The three stage sampling technique was followed to select the sample. In first stage, two major lavender growing blocks (Salooni and Tissa) of Chamba district were selected purposively.In the second stage of sampling, a list of lavender growing villages from each block was prepared and 10 villages were selected randomly from the list. In third stage, a sample of 6 farmers was selected randomly from each

village, thus making the total sample size of 60 farmers. A schedule containing detailed information on various aspects of lavender cultivation such as area under lavender cultivation, input use pattern, labour use pattern, prices of various inputs, quantity of lavender produce and socio-economic characteristics of lavender growers was prepared. The primary data were collected on these well-designed pre-tested schedules by personal interview method and have been analyzed through the tabular method andother mathematical tools using MS Excel.

Economic analysis and financial feasibility: Cost components of lavender cultivation for a period of 10 years were divided into variable and fixed cost. For the establishment of crop in first year, the variable cost included planting material (cuttings), farm yard manure (FYM), bullock charges, human labour and plant protection charges and interest on working capital. The fixed cost included rental value of land, interest and depreciation on fixed capital. For the subsequent years, the variable cost included only human labour charges and FYM charges. Discounted measures were used in feasibility analysis of lavender crop because it required initial investments and have delayed cash flows over the years. The common discounted measures that were used included:

Net present value (NPV): Net present value (NPV) of an investment is the discounted value of all cash inflows and cash outflows of the project during its life time.

$$NPV = \sum_{t=0}^{n} \{(B_t - C_t)/(1+r)^t\}$$

Internal rate of return (IRR): Internal rate of returnis the rate of return at which the net present value of a stream of payments/incomes is equal to zero.

$$NPV = \sum_{t=0}^{n} \{(B_t - C_t)/(1+r)^t\} = 0$$

Benefit cost ratio (BCR): The benefit cost ratio of an investment is the ratio of the discounted value of all cash inflows to the discounted value of all cash outflows during the life of the project.

$$BCR = \sum_{t=0}^{n} \{(B_t)/(1+r)^t\} / \sum_{t=0}^{n} \{(C_t)/(1+r)^t\}$$

Where, B_t = gross returns in time t, C_t = cost in time t , r = rate of interest, t= time period (1, 2, 3……10 years)

Garrett's ranking technique was employed to analyze the constraints faced by the farmers in lavender cultivation.

RESULTS AND DISCUSSION

Economic analysis and financial feasibility: In the establishment phase, lavender cultivation required 19,990 cuttings per hectare. Lavender is a labour intensive crop and labour requirement was highest i.e. 238 man days per hectare in the first year as compared to subsequent years due to high requirement of labour which were carried out only in the first year such as field preparation, and planting.On an average, 21.83 hours of bullock labour per hectare were used for the field preparation purpose.Around 248 quintals of FYM per hectare was used in the year of establishment as well as in the subsequent years. Plant protection chemicals (Bavistin) were used by a few farmers for controlling the fungal diseases (1750 gram per hectare). Second year onwards, only human labour and farm yard manure were used as major inputs in lavender crop. The amount of these inputs varied from year to year, giving an average of 184.38 man days of human labour and 248 quintals of FYM per hectare per year. The proportion of family labour was more than the hired labour in all the years.

The establishment cost included expenses incurred during the first year of cultivation, such as land preparation, planting materials, soil amendments, pest and disease management and labour costs (Table 1). The total cost of establishment of lavender crop was Rs.2.63 lakh per hectare. Among the total variable cost, planting material was the major component contributing about 37 per cent of the total cost @ Rs. 5 per cutting. The rental value of land is included as a component of fixed cost which occupies 18 per cent of the total cost. In second year, the cost of cultivation reduced to Rs.1.32 lakh per hectare and after that it increased at a slow rate up to tenth yearas human labour was used for most of the farm operations and wages of labour tended to increase over the years. On an average, the total cost of cultivation of lavender from second year onwards was Rs.1.59 lakh per hectare per year. The average yield of lavender dried flowers was 3 quintal per hectare. The gross returns from the lavender cultivation varied from Rs. 3 lakh to Rs. 4 lakh over the period of nine years @ Rs.1200 per kg of dry flowers. The net returns from lavender over the years were around Rs. 2 lakh per hectare.

The net value of lavender cultivation in the first year of cultivation at 8, 12 and 15 per cent of discount rates was Rs.9.05 lakh, Rs.7.04 lakh and Rs.5.86 lakh, respectively (Table 2, 3). The BC ratio at discount rate of 8, 12 and 15 per cent was 1.79, 1.73 and 1.68, respectively, which indicated the worthiness of investment on lavender cultivation. The internal rate of returns (IRR) for lavender cultivation was 67 per cent which was considerably higher than the prevalent bank rate which indicated that lavender cultivation offers lucrative opportunities for farmers seeking to maximize profits.IRR greater than the cost of capital indicates a

Table 1. Year wise costs and returns of lavender cultivation on sample households

Particulars	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Variable cost										
Planting material (cuttings)	99950.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Farm yard manure	24800.00	24800.00	29760.00	29760.00	29760.00	37200.00	37200.00	37200.00	49600.00	49600.00
Bullock charges	5457.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hired labour	14337.00	14313.00	14161.00	15046.50	14535.50	16079.00	18332.00	17876.00	19633.50	23008.50
Plant protection (Bavistin)	2800.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Working capital (1+2+3+4+5)	147344.50	39113.00	43921.00	44806.50	44295.50	53279.00	55532.00	55076.00	69233.50	72608.50
Interest on working capital @ 6%	8840.67	2346.78	2635.26	2688.39	2657.73	3196.74	3331.92	3304.56	4154.01	4356.51
Cash variable expenses (6+7)	156185.17	41459.78	46556.26	47494.89	46953.23	56475.74	58863.92	58380.56	73387.51	76965.01
Family labour	57339.00	40611.00	48569.50	48454.00	49805.00	48786.50	55800.00	56256.00	64935.00	61560.00
Total variable cost (8+9)	213524.17	82070.78	95125.76	95948.89	96758.23	105262.24	114663.92	114636.56	138322.51	138525.01
Fixed cost										
Rental value of land	50000.00	50000.00	50000.00	50000.00	50000.00	50000.00	50000.00	50000.00	50000.00	50000.00
Interest on fixed capital @ 10 %	152.57	152.57	152.57	152.57	152.57	152.57	152.57	152.57	152.57	152.57
Depreciation @ 20%	305.14	305.14	305.14	305.14	305.14	305.14	305.14	305.14	305.14	305.14
Total fixed cost (10+11+12)	50457.71	50457.71	50457.71	50457.71	50457.71	50457.71	50457.71	50457.71	50457.71	50457.71
Total cost (A+B)	263981.88	132528.49	145583.47	146406.60	147215.94	155719.95	165121.63	165094.27	188780.22	188982.72
Yield of dry flowers (q/ha)	0.00	2.50	2.50	2.50	3.50	3.50	3.50	3.00	3.00	3.00
Gross returns @ Rs. 1200/kg	0.00	300000.00	300000.00	300000.00	420000.00	420000.00	420000.00	360000.00	360000.00	360000.00
Net returns	-263981.88	167471.51	154416.53	153593.40	272784.06	264280.05	254878.37	194905.73	171219.78	171017.28

profitable investment, while a lower IRR suggests unviability.

Constraints: The lavender growers in Chamba are confronted with various problems, including high cost of marketing, non-availability of human labour, high wages of labour, changing climatic conditions, etc. These challenges not only affect the yield and quality of lavender but also impact the livelihoods of farmers, leading to reduced incomes from its cultivation and increased vulnerability. During the survey conducted among sampled lavender farmers, a range of problems and constraints that they encountered became apparent. These problems and constraints were classified into four different categories viz., input related problems, labour and machinery related problems, marketing problems and others. In the problems related to production, the scarcity of FYM was ranked first because there was a high requirement of farm yard manure for lavender cultivation as the use of chemical fertilizers was limited (Table 4). In problems related to human labour and machinery, high wages of labour was ranked first followed by non-availability of labour.Marketing of the produce was most severe problem faced by the lavender farmers. In the problems related to marketing, first rank was given to the problem of procurement agencies being at longer distance followed by problem in disposal of produce due to lack of specialized agencies and lack of other marketing facilities in the area. There were many other problems related to marketing like high transportation charges, low prices of produce and lack of proper marketing information that negatively affected the cultivation of lavender. The other problems related to lavender cultivation were lack of government support to the farmers, lack of processing facilities, lack of timely transfer of technologies, lack of motivation among the farmers for a different crop and climate change.

Table 2. Year wise cash flows generated in lavender cultivation on sample households (Rs. / ha)

Years	Cost	Gross returns	Net returns
1	263981	-	-263981
2	132528	300000	167472
3	145583	300000	154417
4	146406	300000	153594
5	147215	420000	272785
6	155719	420000	264281
7	165121	420000	254879
8	165094	360000	194906
9	188780	360000	171220
10	188982	360000	171018

Table 3. Financial feasibility of lavender cultivation on sample households

Particulars	Discounting factors(%)		
	8	12	15
Net present value (Rs.)	905470	704698	586090
Benefit cost ratio	1.79	1.73	1.68
Internal rate of return (%)	67	67	67

Table 4. Problems and constraints faced by lavender farmers on sample households (Garrett's score)

Problems	Mean score	Rank
(Garrett's score)		
Production problems		
Scarcity of FYM	65.40	1
High cost of planting material	40.73	3
Non-availability of planting material	59.27	2
Lack of irrigation facilities	34.60	4
Related to labour and machinery		
Non-availability of human labour	60.80	2
High wages of labour	62.97	1
Non-availability of bullocks/machinery	39.7	3
Bullocks/machinery charges are high	36.53	4
Marketing problems		
Disposal of produce is difficult due to lack of specialized agencies	62.77	2
Procurement agencies at longer distances	65.37	1
High transportation charges	40.8	4
Lack of marketing facilities	61.13	3
Low prices of produce	40.00	5
Lack of marketing information	29.93	6
Others		
Lack of timely and appropriate transfer of technology	32.50	5
Lack of government support	62.33	1
Climate change	42.83	4
Lack of motivation among farmers for a different crop	59.17	2
Lack of processing facilities	53.17	3

CONCLUSION

Lavender cultivation can be a profitable venture with BC ratio of more than unity and considerably high internal rate of returns (67%).To unlock the full potential of lavender crop, the government should prioritize providing adequate marketing facilities to lavender farmers. To enhance the productivity and profitability of lavender farming, it is imperative to transfer modern knowledge and best practices to farmers. The government and private sectors should invest in distillation units for essential oil extraction, drying and packaging

facilities, extraction units for lavender honey and wax, and manufacturing units for lavender based cosmetics and pharmaceuticals.

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