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Energy Regulatory and Industrial Complex (ERIC): A New Framework

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***Abstract*:** Climate change agreements force us to explore interconnected social, economic, and energy related transitions needed to combat the effects of anthropogenic activities on the Earth's climate. To help us understand what brought us to where we are today and guide us in our decision-making for tomorrow, we introduce the Energy Regulatory Industrial Complex model (ERIC). ERIC illustrates the transformation of energy from energy sources through to energy services, as in energy flow diagrams, but adds the emissions greenhouse gases from those transformations. ERIC includes the creation of wealth enabled by energy services, innovation that supports advances in science and technology, and activities that maintain and sustain existing systems. Policies and geo-politics can influence each decision and investment stream. At its core, ERIC relies on a firm foundation of education. Using data from the United States and India, we show how ERIC can be used to pinpoint the vulnerabilities and places for interventions in transitioning to a clean energy future.

***Keywords*:** Climate change, Education, Energy flow diagram, Energy systems, Energy transition

For decades now we have been hearing about the increases in atmospheric concentrations of greenhouse gases that blanket the earth, trapping heat, and altering the patterns of rain- and snowfall, storm intensities, land and sea surface temperatures, glacial melt rates, and the timing of seasonal changes. Those involved in agriculture have witnessed declining yields, the increased prevalence of invasive weeds, and an influx of pests and pathogens adapted to the new (often warmer and more humid) conditions on the ground (Malhi et al., 2021). Extended droughts and desertification of land or more severe storms and floods have destroyed crops in the field (Arora 2019). While yields and growth rates of crops like rice and wheat may increase in the carbon dioxide-rich atmosphere of the present and future, the nutrient production of these crops under high CO_2 concentrations tends to decrease (DaMatta et al., 2010, Leisner 2020). One thing is clear: climate change has not left and will not leave agriculture unscathed. In a broader sense, the places in which we live are being fundamentally altered by climate change. That then leads us to question: "What has been or is being done to mitigate the impacts of climate change or adapt to those impacts?"

Recall that in 1997, the United Nations Framework Convention on Climate Change (UNFCCC) adopted the Kyoto Protocol. The Protocol called for industrialized countries (the Annex I Nations) to limit and/or reduce emissions of anthropogenic greenhouse gases causing climate change by an average of five percent relative to 1990 levels during the first commitment period of 2006-2012 (United Nations Climate Change; UNFCCC, 1997). Under the Protocol, much of Europe, North America, the Russian Federation, and Australia needed to reduce emissions, but China and India did not. By 2022, 192 nations had ratified Kyoto. Notably, Canada, a large producer of oil shale, withdrew in 2012, and although U.S. President Bill Clinton signed the Kyoto Protocol, he made it clear he would not submit it to the United States Senate for ratification unless key developing countries also participated (U.S. Department of State 1998). Thus, although President Clinton signed the Protocol on behalf of the country, the Senate never ratified it, and the United States was not bound by its commitments.

As Figure 1 below illustrates, despite ratification/acceptance/approval of the Kyoto protocol by no fewer than 55 parties to the United Nations Framework Convention on Climate Change (UNFCCC) by 2005 (Status of Ratification, n.d.), emissions continued to climb in most regions of the globe, but particularly in Asia.

By 2015 and the 21st Conference of Parties meeting in Paris, focus shifted from limiting emissions by industrialized nations to a global effort decrease greenhouse gas concentrations. Accelerated action was required to maintain the average global temperature rise to below 1.5 °C, or, more likely, below 2°C by the end of the 21st century (Article 2). The new mechanism created for achieving that goal: the Nationally Determined Contributions (NDCs, Article 4). Based on the "principle of . . . common but differentiated responsibilities and respective capabilities" (Paris Agreement, p. 1), and recognizing the differences in financial and technological abilities among nations, every party to the 21st Conference would submit plans for reducing emissions from burning fossil fuels and mitigating climate change and would provide updates every five years. Key to the success of the Paris Agreements would be financial flows from the developed countries to those with fewer financial resources, technology transfers, and capacity building where needed (Articles 8 - 11). The Paris Agreement recognized the need to safeguard food security and the “particular vulnerabilities of food production systems to the adverse impacts of climate change” (Paris Agreement, p. 1). It also affirmed the importance of education and training in achieving its goals. Thus, rather than just focusing on the quantifying emission levels, the Paris Agreement forced a reckoning with interconnected social, economic, and energy systems in combatting the effects of climate change.

With so much at stake and so many moving pieces at global, national, and subnational scales, it behooves us to develop ways to think about these systems, to diagram them, to create a map that can help us make sense of this amalgamation, to help us understand what brought us to where we are today, and guide us in our decision-making for tomorrow. To that end, we introduce the Energy Regulatory Industrial Complex model (ERIC) (Fig. 2). In the balance of this paper, we explore ERIC and its value in understanding the complexities of energy systems and decision-making about energy. Using data from the United States and India, we show how ERIC can also pinpoint the vulnerabilities and places for interventions in transitioning to a clean energy future.

The Value of the Energy Regulatory Industrial Complex model

To understand ERIC and its value for examining the connections between agriculture, energy, and climate change, we will begin in the lower left of Figure 2 with the box labeled “Primary Energy Sources.” Furthermore, to illustrate our points we draw on the examples of our host country, India, and our home country, the United States.

Currently, there are nine primary energy sources available for our use: (1) solar radiation, (2) nuclear now fueled by uranium or a plutonium and uranium mix (MOX), (3) hydropower (falling water), (4) wind, (5) geothermal heat, (6) natural gas, (7) coal, (8) biomass (including firewood and ethanol made from corn or bagasse), and (9) petroleum. We feed these raw sources into refining processes then transform them into forms of energy that can be tapped to provide the services we rely on for our daily existence (Fig. 3).

Note that the width of the bars and the size of the boxes in the flow charts give us an easy way to determine the relative amounts of each of the inputs and the corresponding uses by the residential, commercial, industrial, and transportation

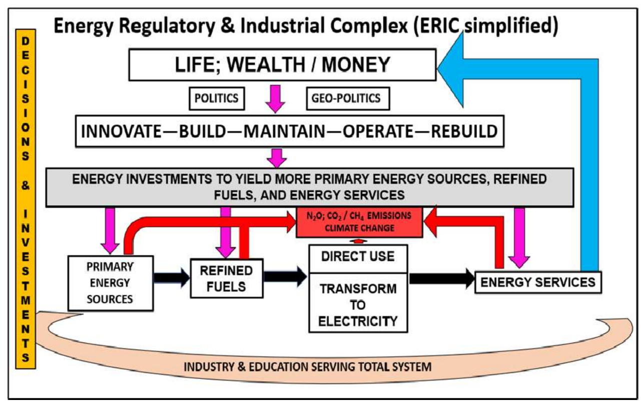

Fig. 2. Simplified Energy Regulatory Industrial Complex model (ERIC)

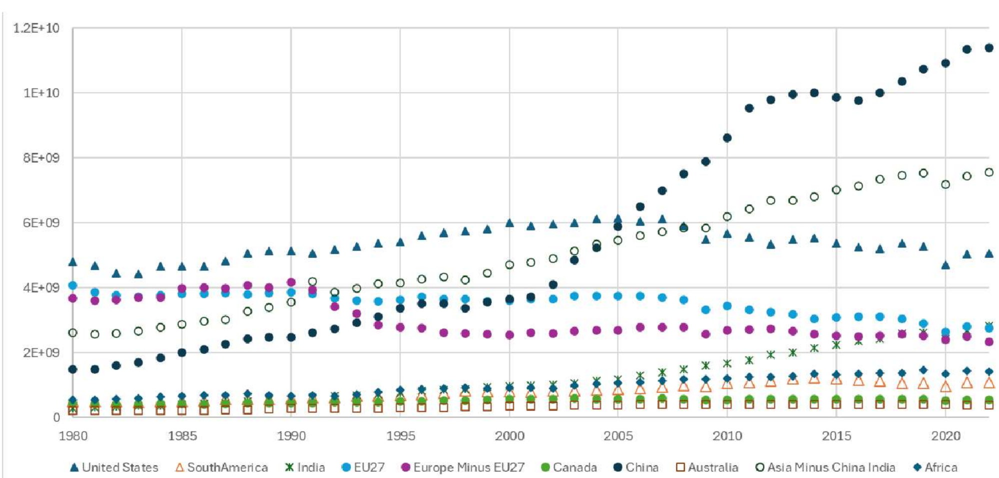

Fig. 1. Annual Greenhouse Gas Emissions, tonnes, from industry and burning fossil fuels but not including land use changes, shipping or air transportation (Source: Based on data from Our World in Data, https://ourworldindata. org/co2-emissions)

sectors. For both the United States and India, about 1/3 of the input energy provides useful energy services such as lighting, heating, cooling, or powering machinery, while 2/3 gets rejected as heat due to the inefficiencies of our conversion processes. In India, coal contributes over 43% of the energy used whereas in the United States coal amounts to 9.3% of the energy mix. Conversely, in the United States, natural gas now supplies over one third of the energy (35.7%) but only 5.7% in India.

Agriculture falls into the "Industrial" category in the Energy Flow Diagrams. In the United States, the agriculture sector relies on natural gas and liquid petroleum gas for heating and drying grain (Hitaj and Suttles 2016). Diesel and gasoline power farm machinery and electricity flows to lights, cooling systems, and pumps. In India, coal contributes to agriculture indirectly through electrical generation and as a feedstock to the production of nitrogen fertilizer. Coal enters agriculture directly in the form of fly ash, a powdery residue of the combustion of coal. Adding fly ash to soils can decrease their bulk density and aid in moisture retention (Yousuf et al., 2020) The lime in fly ash also reacts with acidic elements in soils, allowing for the release of nutrients and aiding in the remediation of severely degraded soils (Reid 2022, Yousuf et al., 2020).

Moving further along the bottom of the model brings us to energy services. The visible and yet unremarkable products of energy, energy services are the hallmarks of modern life: our ability to flip switches to turn on computers, lights, and water pumps; to traverse state or continents, or harvest grains more efficiently than using human or animal power; to access communications networks that reach neighbors, markets, or colleagues half a world away. To understand the emissions from the energy systems (Fig. 5), their contribution to climate change, we need to turn to a different type of modelling approach.

Using Life Cycle Analysis (LCA), we can focus on a particular technology or set of technologies (the "functional units"), accounting for environmental impacts throughout their expected life (Fig. 6).

Drawing the boundaries of the analysis is key to any life cycle or "cradle to grave" analysis. The boundaries set the scope of the analysis, helping researchers identify which processes and flows to include and which have no bearing on the question of interest (Hertwich et al., 2015, Nugent and Sovacool 2014). People making claims of carbon free electrical generation often isolate the generation plant from the upstream and downstream flows, draw the boundary so as to eliminate construction or decommissioning impacts from consideration, and focus only on emissions while the plant is generating electricity.

Maintaining the cradle to grave approach, we then explore environmental harms associated with resource extraction, processing, and fabrication of fuels and parts for the technology of interest. An LCA can encompass mining for non-renewable resources such as copper for wiring, lithium for batteries, chromium, and nickel (used in wind turbines).

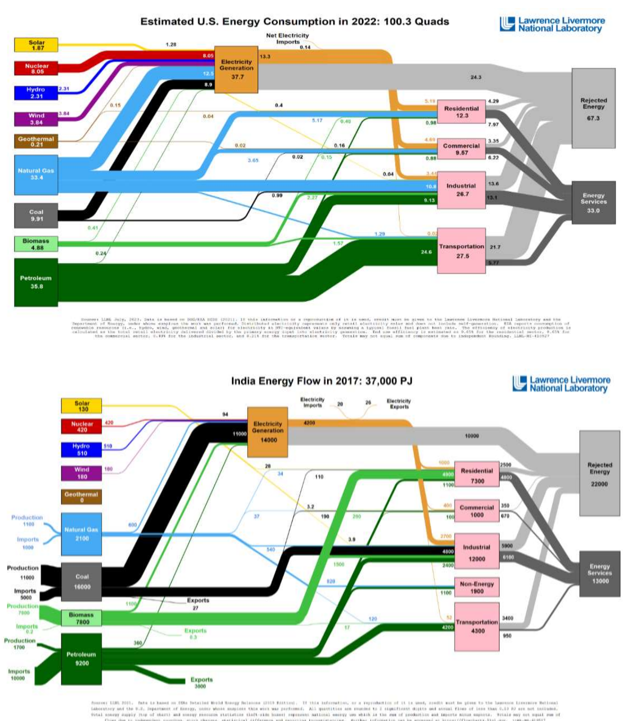

Fig. 4. Lawrence Livermore National Laboratory Energy Flow Diagrams for the (a) United States (2021) and (b) India (2017); One quad = 1.055 EE18 Joules and 1 PJ = 1.0 EE15 Joules

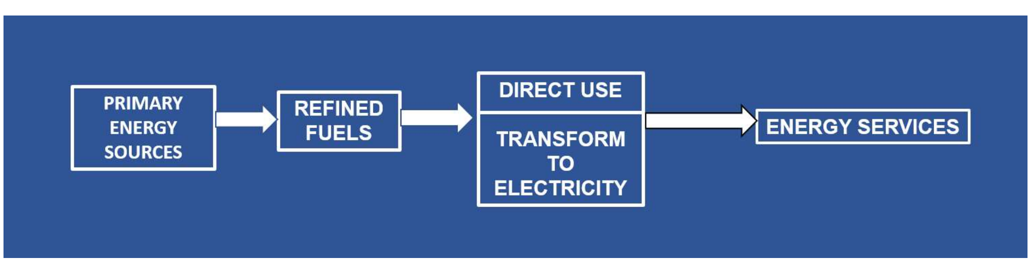

Fig. 3. Transforming primary energy (Sources into Modern Energy Services)

LCA can include emissions associated with transportation to a construction site and transformation of that site to one suited to its new purpose. Operation and maintenance of the energy facility often leads to emissions of particulate matter and emissions of carbon dioxide, sulfur and nitrogen oxides that could be incorporated into an LCA. In thermal systems, water becomes steam that spins turbines to generate electricity. Steam may escape into the atmosphere or may condense to be reused. Water flowing back to rivers or lakes may carry with it particulate matter or radioactive particles from the plant or reactor. Water also may have increased in temperature, impacting the delicate ecosystems beyond the discharge point. Finally, decommissioning or disposal of the facility carries its own environmental price tag as it gets cocooned and cordoned off, as happens with nuclear power plants, or remediated and used for other purposes. In the end, the LCA can provide us with summaries of the various inputs and outputs of each of the stages of life of the facility as well as the total throughout the life of technologies.

Drawing on the example of coal-fired electrical generation and based on a very detailed analysis of the flows of materials and energy through this system, Spath, Mann, and Kerr determined that over 97% of CO_2 emissions occur during power production (996 g/kWh for the average coal fired plant). Power production also resulted in the emission of 3.35 g/kWh of nitrogen oxides (NO_2) and 6.70 g/kWh of sulfur oxides (SO_2), both components of air pollution and smog. In contrast, 99% of the methane production occurred during the mining of the coal-equating to 0.913 g/kWh. Sequestration of methane releases during mining will be vital to reducing the impact of that potent greenhouse gas. Indeed, carbon dioxide, methane, and NO_x are some of the components of the red Climate Change box in the ERIC model.

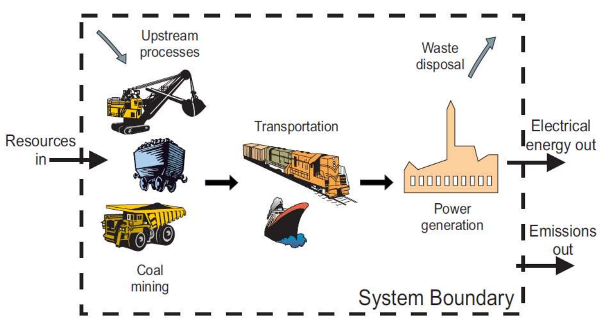

Fig. 6. Systems boundaries for a life cycle analysis of a coal fired power plant (Source: Spath, Mann, and Kerr, p. 4)

We can compare the LCAs of various electrical generation technologies on a per unit energy produced to understand their contributions to this aspect of environmental degradation. Figure 7 shows the output of numerous life cycle analyses for common electrical generation technologies, per kWh of electricity produced (NREL, p. 2). Not surprisingly, emissions associated with production from fossil fuel fired plants, even with carbon capture and storage, exceed the life cycle greenhouse gas emissions of production from renewable energy technologies.

Missing from both the energy flow diagram and the LCA are factors that give rise to selection of resources used, auxiliary benefits of particular technologies (such as heat generation or the creation of water reservoirs for irrigation (Weisser 2007)) and contextual conditions that influence decisions about technologies and investments, such as where to site an electrical production facility and whether to invest in solar or coal-fired generation. These models also fail to account for the recursive nature of the systems, the ongoing need for investments in resources, upgrades to equipment, education of personnel, and response to changing policies and demand profiles. Western economic models, built on the examples of the post-agricultural world in which economies of scale and increasing centralization brought lower costs and higher profits, tended to view "progress" and development in a unidirectional fashion. Progress meant greater amounts of goods and services offered for sale in larger and larger marketplaces, greater wealth, and thus greater prosperity (Ekelund and Hebert 2014, Lefeber 2000, North and Thomas 1970). That legacy has carried over into models like the energy flow diagram and the LCA.

However, we know that governments and legislators do step in to help move economies in desired directions when markets do not achieve desired results (the "Politics" box in Figure 2). In the case with renewable energy adoption and

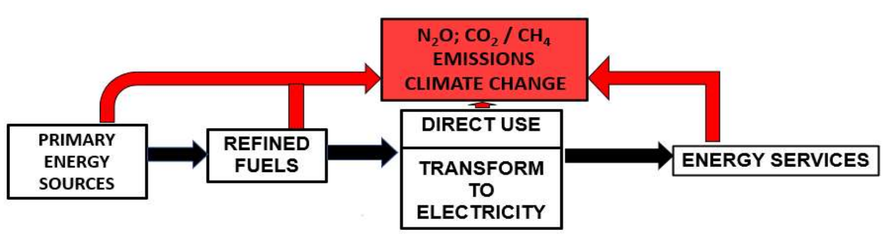

Fig. 5. ERIC, highlighting the emissions driving climate change

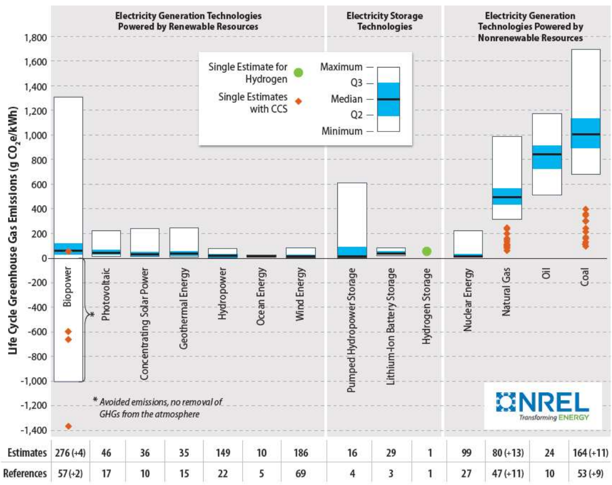

Fig. 7. Comparative life cycle greenhouse gas emissions from electrical generation technologies, gCO_2 e/kWh (Source: National Renewable Energy Laboratory, Life Cycle Greenhouse Gas Emissions from Electricity Generation: Update, p. 2)

solar photovoltaic (PV) panels, for example, initial success in the United States has been attributed to the adoption of state-level Renewable Portfolio Standards (RPS) that required utilities to include specified percentages of renewable energy in their resource portfolios (Bozuwa, Mulvaney, Estevez, Karlsson, and Malhotra, 2024). The California Solar Initiative, enacted in 2006, spurred investments in residential solar with rebates to customers of Pacific Gas and Electric, Southern California Edison, and San Diego Gas and Electric (Mendes 2022). The goal of 1940 MW of new solar capacity was reached two years ahead of schedule. President Obama's American Recovery and Reinvestment Act (ARRA) of 2009, which provided tax credits for solar technologies, really focused attention on the technology and its adoption. Figure 8 below shows the cumulative installation of solar PV across the United States, from a near negligible amount prior to the enactment of ARRA to the present. As the adoption of solar PV expanded, the economies of scale in producing solar panels helped reduce prices by more than half for residential

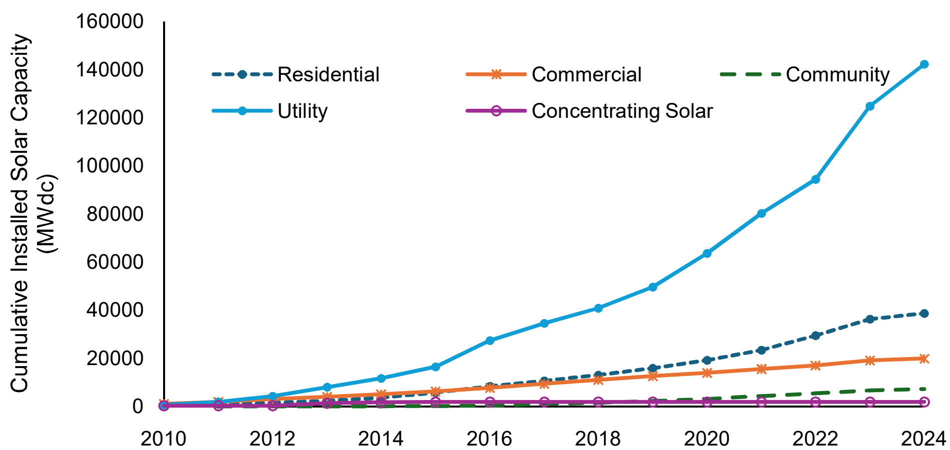

Fig. 8. Cumulative solar capacity in the United States 2010 through the first half of 2024, MWdc (Data Source: Solar Energy Industries Association, Solar Industry Research Data)

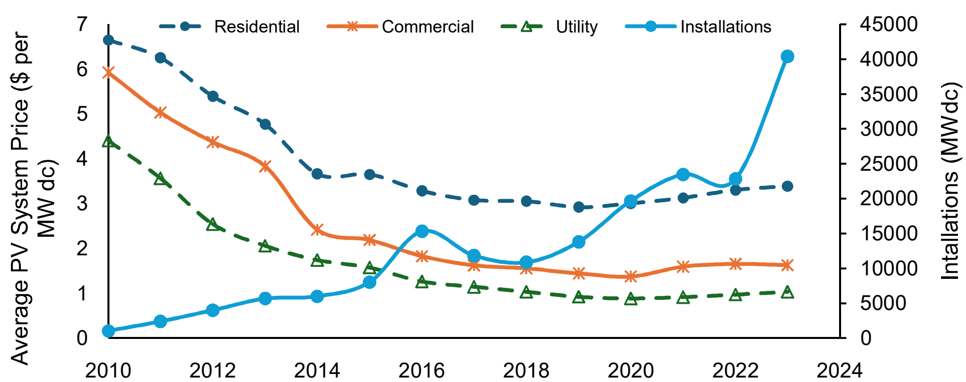

Fig. 9. Average Solar Photovoltaic (PV) System Price and Numbers of Installations, 2010 through 2023 (Data Source: Solar Energy Industries Association, Solar Industry Research Data)

customers and about 75% for commercial and utility-scale customers, further stimulating investment (Fig. 9; see also Gerarden 2018).

While many of us think of solar installations on rooftops as serving the needs of those dwelling or working in the building below, utility-scale solar parks have become increasingly common as nations strive to meet decarbonization goals. India's Bhadla Industrial Solar Park in Rajasthan, covering 5,700 hectares of the Thar desert, has a capacity of 2,245 MW (Dauphin 2022). This "ultra mega" solar installation can be seen from space (Fig. 10). One challenge for any facility of this size will be to ensure the electricity generated reaches awaiting customers through modernized transmission and distribution networks. Additionally, only time will tell what impact the construction of the arrays and shading of such a large swath will have on the desert ecosystem below and around it.

Fig. 10. Satellite Imagery of the Bhadla Solar Park, Rajasthan, India, 26 January 2022. (Source: Lauren Dauphin, https://earthobservatory.nasa.gov/images /149442/soaking-up-sun-in-the-thar-desert)

As the example of solar PV demonstrates, we can no longer assume static or equilibrium models, or even models that assume progress towards some nebulous goal of better and more, but instead need to think in terms of models that capture change over time and place. We need models that allow us to reflect the complexity of the system of interest sufficiently so that we can identify the important "leverage points." According to systems theory, leverage points provide opportunities for change (Meadows 2008). They are "points of power" where small changes can ramify through the system to result in large shifts (Meadows, p. 145). Our question then becomes, "Where are the leverage points in the Energy-Regulatory-Industrial-Complex that will accelerate the transition to a clean and renewable energy future?"

ERIC Points Towards a Clean Energy Future

ERIC enables us to follow the impact of policies enacted at the federal, regional, state or even the local level, on pathways of innovation, decisions to invest in new technologies or to maintain existing systems (recall the top portion of Fig. 2). For example, while the government of India has espoused programs to bolster the growth of clean energy, it continues to subsidize fossil fuels, in part by

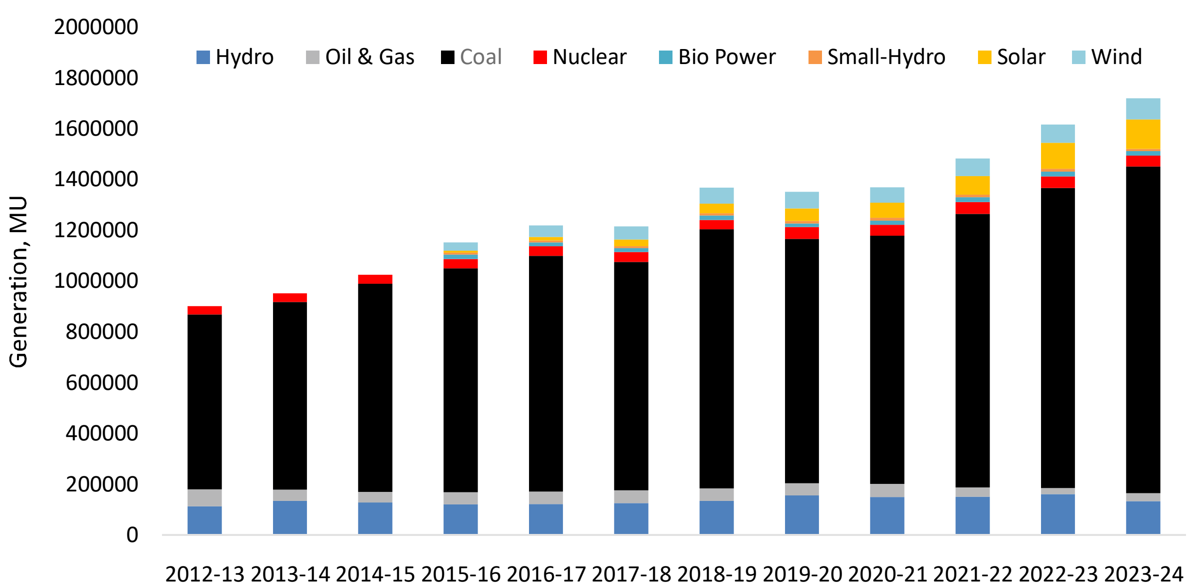

Fig. 11. Electricity Supply in India through May 2024 (Source: India Climate and Energy Dashboard, https://iced.niti.gov.in/energy/electricity/generation/power-generation)

capping their prices rather than letting supply and demand (i.e. market forces) set prices. In FY 2023, “clean energy subsidies remained less than 10%, while subsidies for coal, oil, and gas contributed around 40% of total energy subsidies” (Raizada et al., 2024). In the electricity sector in 2023, coal subsidies reached INR 50,000 crore (USD 6.2 billion) while subsidies for renewable energy installations amounted to only INR 14,843 crore (USD 1.8 billion). In July of 2024, the Modi government requested that power companies fast track equipment investments for about 31 GW of new coal-fired electrical generation (Singh 2024). That figure dwarfs the 2 – 3 GW added annually over the past decade (Fig. 11). To cover the expected growth in electrical demand--due in part to a need for cooling in response climate change driven heat increases--and to be able to fulfill campaign promises of stable electric supplies, Modi and the BJP party are backing coal rather than investing those funds in renewable sources and battery storage solutions (Arasu 2024, Mathur 2024).

While caps on the prices of fossil fuels in India keep the prices low for those who cannot afford high prices, the rural poor and small farmers, those price caps lead to a continued dependence on fossil fuels rather than an expansion of renewable energy supplies. We also know that the rail lines in India ship coal from the mines to the electric generating facilities (Adhikari 2024). The revenues from those shipments subsidize rail fares for human passengers. A push to phase out the use of coal could impact about 23 million people who ride the trains daily (Times of India 2023). In systems language, price caps and the revenues from coal transported by rail serve as balancing or stabilizing feedback loops-loops that push back against change from the current state (Meadows, pp. 28-30) (Fig. 12).

In the United States, the bi-partisan Inflation Reduction

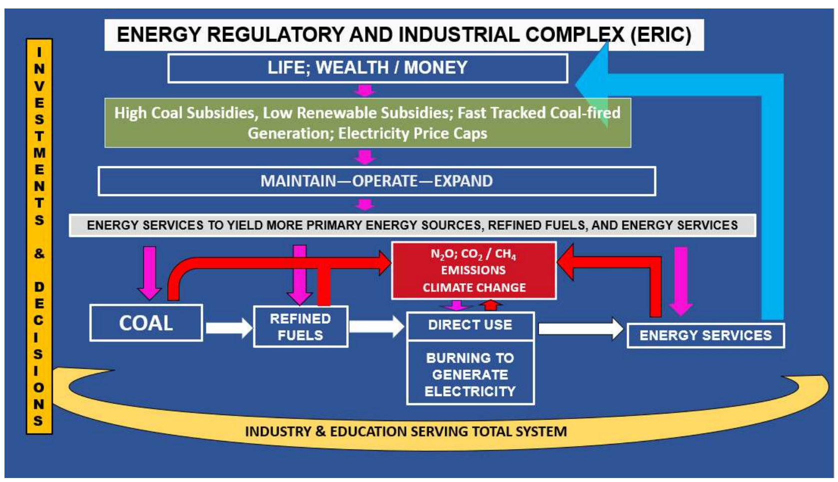

Fig. 12. An overview of the Indian coal economy as captured by ERIC

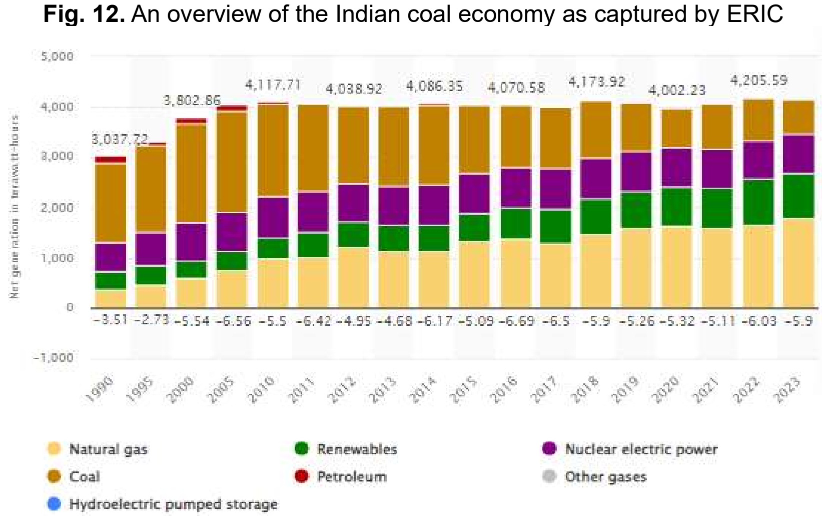

Fig. 13. Electrical Generation in the United States, tWh (Source: Statista 2024) (Note: Negative numbers at the bottom of the figure represents the contribution of hydro/pumped storage)

Act (IRA) of 2022 provided investment or production tax credits for those investing in solar or wind technologies, energy from municipal solid waste, geothermal electric programs, and tidal or hydrokinetic energy (U.S. EPA, 2024). Investment tax credits were also available for fuel cell technologies, combined heat and power, micro-turbines, micro-grid controllers, and energy storage technologies. Tax credits also spurred sales of electric vehicles, with 1.4 million new electric vehicles registered in 2023, 40% more than in 2022 (International Energy Agency, 2024). Unfortunately, fossil fuel companies, particularly oil and gas firms, still benefit from tax credits for continued pumping out of marginal wells, low lease rates on public lands, tax credit for investments in carbon capture and sequestration projects, and sales tax exemptions. The International Monetary Fund estimated that fossil fuel subsidies amounted to about $757 billion in 2022 (Brind'Amour 2024). Despite the focus on a cleaner future, Americans remain addicted to fossil fuels, as shown in Figure 13.

To de-carbonize our energy future, we tend to think first of policy-based leverage points. However, to achieve economic growth without destroying the environment will take a much more concerted effort at enacting policies that redirect investment away from fossil fuels and towards solar thermal and photovoltaic facilities, like the Cochin International Airport in Kerala (Lombard Odier 2024) or the almost 15 GW of utility scale solar systems that came on-line in the first half of 2024 (Gupta 2024). It will require on- and off-shore renewable energy developments, and new, as yet undiscovered technologies. It will demand a firm financial and technological commitment to clean, renewable energy countrywide, for electrical production, industry, and transportation. It also will necessitate agricultural practices that don't depend so heavily on fossil-fuel derived fertilizers and pesticides, and fossil fuel powered equipment.

To accomplish all of that we need to focus on breaking down the biggest barrier to a clean energy future: the mindset that gave rise to the large scale, centralized, fossil fuel dominated energy systems on which we now depend. We need to start by questioning the very premises on which that system relies, such as the “need” for endless supplies of cheap material goods and instantaneous satisfaction of personal wants. We need to think about the sources of our energy and the financial, social, and environmental costs associated with them. And then we must act.

Recall that at the base of the ERIC model lies a vital key to change: education. The students of today will be the decision-makers of tomorrow. We must teach them about these systems and the impacts on the environment--like the drawdown of groundwater supplies and the climate changing emissions of greenhouse gases. We need to instill in them the value of preserving the earth today so that the people of tomorrow have adequate supplies of nutritious food to eat. We must use tools like ERIC to continue engaging with students, exciting their creativity, spurring them to question what was once taken for granted, and ensuring they are suitably equipped to face the challenges of the future.

CONCLUSION

I am reminded of the words of Eleanor Roosevelt:

Surely, in the light of history, it is more intelligent to hope rather than to fear, to try rather than not to try. For one thing we know beyond all doubt: Nothing has ever been achieved by the person who says, 'It can't be done.'

The Energy Regulatory Industrial Complex model (ERIC) allows us to visualize the interconnected elements that comprise the world's energy systems. ERIC allows us to understand the factors that have led to our current energy dilemmas. ERIC also helps us make informed decisions about policies; investment in technology, innovation, and education; and how we will confront the challenges presented by our changing climate. Armed with ERIC, it can be done.

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Comprehensive Review of Conodonts: Evolution, Biostratigraphy, Paleo-environment, and Economic Significance

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***Abstract*:** This paper synthesizes the evolutionary history, biostratigraphic utility, paleo-environmental significance, and economic applications of conodonts into a single comprehensive document. In biostratigraphy, conodonts are indispensable markers that enable precise dating and correlation of rock layers across geological time. Their evolution, distribution, and distinct utility as index fossils offer profound insights into Earth's historical timeline, illuminating evolutionary patterns and unveiling past life forms. Moreover, conodonts serve as sensitive proxies for paleoceanography and paleoclimate, enabling nuanced reconstructions of ancient environments and climatic fluctuations. Their microscopic yet informative nature provides a window into past ecosystems, contributing to a deeper understanding of Earth's environmental evolution. Conodonts also wield substantial influence in today's economy. Industries such as petroleum exploration benefit significantly from their utilization, particularly through the Conodont Color Alteration Index (CAI) and thermal maturity characteristics. These traits aid in assessing thermal maturity in rocks, facilitating resource identification and refining exploration strategies. This integrative approach not only underscores the multifaceted importance of conodonts but also paves the way for future interdisciplinary research and application.

***Keywords*:** Conodonts, Biostratigraphy, Paleo-environment, Index fossils, Paleoceanography, Petroleum exploration

The first discovery of conodonts dates to Heinz Christian Pander's findings in 1856 (Pander 1856). The name "pander" frequently appears in the scientific nomenclature of conodonts (Donoghue and Purnell 1999). However, it wasn't until the early 1980s that the first fossil evidence of the entire animal was uncovered, shedding light on the soft tissues. Subsequently, discoveries in the 1990s in South Africa revealed exceptionally preserved fossils, showcasing even muscle fibers. This discovery definitively classified these organisms as primitive vertebrates (Sweet and Bergström 1995).

Conodonts, the extinct jawless vertebrates known primarily through their tooth-like elements, have played a significant role in understanding early vertebrate evolution and paleoenvironments. Conodonts were not vertebrates in the traditional sense of possessing a backbone or vertebral column. Instead, they were early chordates, belonging to a diverse group of extinct marine organisms characterized by having a notochord at some stage in their life cycle. Therefore, conodonts, while lacking true vertebral elements, exhibit anatomical features typical of early chordates, such as a notochord, leading to their classification as early relatives or precursors to vertebrates rather than true vertebrates themselves (Purnell et al., 2020). First appearing in the Cambrian period around 500 million years ago, conodonts are predominantly recognized through their small, apatite-composed elements. The discovery of soft-tissue fossils in the 1980s confirmed conodonts as eel-like creatures with intricate feeding mechanisms, supporting earlier hypotheses about their feeding apparatus (Sweet 1988, Aldridge et al., 1986). This discovery provided a clearer picture of their morphology and ecological roles.The evolutionary trajectory of conodonts spans from their simple early forms in the Cambrian to their diversification and complexity in the Ordovician through Devonian periods, and finally to their decline and extinction by the Late Triassic (Sweet 1988, Donoghue and Purnell 1999). Their evolution is marked by significant morphological innovations, particularly in their feeding apparatus, which adapted to various marine environments and ecological niches (Purnell et al., 2000). This adaptability is reflected in the fossil record, where conodont elements serve as important biostratigraphic markers, helping to divide geological time into distinct biozones (Sweet 1988).

Conodonts are not only significant for their evolutionary and biological insights but also for their utility in biostratigraphy and paleoclimatology. Their widespread distribution, rapid evolutionary changes, and distinct morphological features make them excellent index fossils for dating and correlating sedimentary rock layers (Donoghue and Purnell 1999, Purnell et al., 2000). Additionally, conodont elements have been used to infer past ocean temperatures and environmental conditions, contributing valuable data for reconstructing paleoclimates (Grossman 2012).

The association of conodonts with other fossil groups further enhances their importance in paleoecological studies.

Their co-occurrence with brachiopods, trilobites, corals, bryozoans, ammonoids, nautiloids, radiolarians, and foraminifera provides insights into ancient marine environments and helps in dating and correlating marine sedimentary rocks across different regions (Miller 1984, Aldridge et al., 1986, Sweet 1988, Clark et al., 1981).

This review paper employs a comprehensive approach to synthesize existing published literature on conodonts, focusing on their evolution, biostratigraphy, paleoenvironmental significance, and economic applications.

MATERIAL AND METHODS

In this review, the methodology involved systematic searches of scientific databases of conodonts, including but not limited to PubMed, Web of Science, and Google Scholar, to identify relevant peer-reviewed articles, books, and conference proceedings. Search terms such as "conodont evolution," "conodont biostratigraphy," "conodont paleo-environment," and "conodont economic significance" were utilized to ensure a thorough retrieval of pertinent literature. Inclusion criteria encompassed studies that provided substantial insights into the mentioned aspects of conodont research. The selected literature was critically reviewed, analyzed, and synthesized to construct a cohesive narrative highlighting the multifaceted significance of conodonts. Emphasis was placed on elucidating key evolutionary trends, bio-stratigraphic utility, paleo-environmental proxies, and the practical implications of conodont research in modern industries, particularly in petroleum exploration. Through this methodology, the paper aims to offer a comprehensive overview of conodonts' importance and their role in advancing our understanding of Earth's geological history.

Origin and Evolution of Conodonts

The earliest conodonts appear in the fossil record during the Cambrian period, approximately 500 million years ago (Sweet 1988). These early forms are primarily known through their conodont elements, which are small, tooth-like structures composed of apatite (calcium phosphate). The origin of conodonts remains somewhat mysterious because, being soft-bodied, only their tiny, tooth-like structures are usually preserved. Early hypotheses suggested that conodont elements were parts of the feeding apparatus of a larger organism. This was confirmed when soft-tissue fossils were discovered in the 1980s, revealing conodonts to be eel-like creatures with complex feeding mechanisms (Aldridge et al., 1986).

Conodonts underwent significant evolutionary changes from their first appearance in the Cambrian to their extinction in the Late Triassic, approximately 200 million years ago. Their evolution is marked by the diversification of conodont elements, which became more complex and varied over time. These changes are well-documented in the fossil record and are used to divide geological time into conodont biozones. The earliest conodont elements are simple and consist of cone-shaped structures. These forms are typically categorized within the Protoconodontida and Paraconodontida groups. The simplicity of these early conodonts suggests they were among the first vertebrates to develop hard tissues (Sweet 1988).

During the Ordovician period, conodonts diversified rapidly. This period saw the emergence of complex conodont apparatuses, which included a variety of element types such as pectiniform and ramiform. The evolution of these apparatuses likely reflects changes in feeding strategies and ecological niches (Donoghue and Purnell 1999). Conodonts reached their peak diversity during the Silurian and Devonian periods. The development of complex, multi-element apparatuses continued, and conodonts adapted to various marine environments. The Devonian period, in particular, was a time of significant morphological innovation for conodonts, correlating with the diversification of marine ecosystems (Purnell et al., 2000).

The diversity of conodonts remained high during the Carboniferous and Permian periods. Conodont elements from these times show considerable variability, suggesting a wide range of ecological adaptations. This period also saw the development of sophisticated conodont apparatuses, including those with specialized grasping and cutting elements (Aldridge et al., 1986). Conodonts began to decline in diversity during the Triassic period, eventually becoming extinct by the end of the Late Triassic. The reasons for their extinction are not entirely clear, but it is likely linked to the dramatic environmental changes and mass extinctions occurring at the time (Sweet 1988).

The anatomical study of conodonts has revealed much about their functional morphology and feeding mechanisms. Early conodonts had relatively simple feeding structures, but over time, they evolved complex apparatuses consisting of multiple element types. These elements likely served different functions within the conodont's mouth, such as grasping, slicing, and grinding food (Donoghue and Purnell 1999). The discovery of well-preserved conodont fossils has shown that they were soft-bodied, eel-like creatures with large eyes and a notochord. The arrangement of their elements suggests that conodonts used a complex feeding apparatus, similar in some respects to the teeth of modern vertebrates (Aldridge et al., 1986).

Conodonts are highly significant in paleontology due to their use in biostratigraphy. Their widespread distribution, rapid evolutionary rates, and distinct morphological changes over time make conodont elements ideal for dating and correlating sedimentary rock layers. Conodont biozones are used worldwide to delineate geological time periods, particularly in the Paleozoic era (Purnell et al., 2000).

Association with Other Fossils

Conodonts are often found in association with various other fossil groups, which is significant for understanding paleoecology, depositional environments, and biostratigraphy. Conodonts frequently co-occur with brachiopods and trilobites, especially in Cambrian and Ordovician strata, helping reconstruct ancient marine environments as these organisms typically inhabited similar ecological niches (Miller 1984). During the Silurian and Devonian periods, conodonts are commonly found alongside corals and bryozoans, indicating reef and shallow marine environments, and aiding in dating reef structures and understanding their development (Aldridge et al., 1986). In the Carboniferous and Permian periods, conodonts are often found with ammonoids and nautiloids, providing valuable biostratigraphic markers for correlating marine sedimentary rocks across different regions (Sweet 1988). In deeper marine settings, conodonts are associated with radiolarians and foraminifera, helping interpret deep-water depositional environments and oceanic conditions (Clark et al., 1981). Conodonts of Triassic age were found associated with ammonites, foraminifera and other fossils in the Kumaun Region of Tethys Himalayas (Sahni and Prakash 1973, Mishra et al., 1973, Chabra and Mishra 1999).

Conodonts as Index Fossils

Conodonts are considered excellent index fossils due to several key attributes that make them invaluable for biostratigraphy. Conodonts were globally distributed across various marine environments. Their widespread presence allows for correlation of rock layers over vast geographic areas (Sweet 1988). Conodonts evolved rapidly, with distinct species appearing and disappearing over relatively short geological timescales. This rapid turnover provides high-resolution biostratigraphic markers, enabling precise dating of rock sequences (Donoghue and Purnell 1999). Conodont elements have unique and easily recognizable morphologies. This distinctiveness facilitates their identification and differentiation from other microfossils, ensuring accurate biostratigraphic correlations (Purnell et al., 2000). Conodonts are commonly found in marine sedimentary rocks, often in large numbers. Their abundance makes them reliable indicators for biostratigraphic studies, even in small sample sizes (Aldridge et al., 1986). Conodonts inhabited a wide range of marine environments, from shallow coastal areas to deep ocean basins. This ecological versatility enhances their utility as index fossils across diverse sedimentary settings (Clark et al., 1981).

Conodonts as key index fossils played a crucial role in determining the age of rocks across various geological periods. In the Cambrian period, Protohertzina anabarica is found in the middle Cambrian strata of Siberia and South China, and it is used for zoning in the Anabarites–Protohertzina Zone (Dong et al., 2001). During the Ordovician period, species like Streptograptus gracilis help define the early Ordovician Tremadocian stage in the stratigraphy of Wales and England (Williams 1982), while Protopanderodus rectus is an important index fossil for the Middle Ordovician Caradoc stage in Estonia and Wales (Lindström 1971). Additionally, Baltoniodus triangularis serves as a guide fossil in the uppermost Ordovician strata, particularly the Ashgill series (Lindström 1971).

In the Silurian period, Pterospathodus amorphognathoides is an index fossil for the Lower Silurian Aeronian stage in British stratigraphy (Loydell 1992), and Oulodus eurekensis is used as an index fossil for the Silurian-Devonian boundary in North America (Murphy and Valenzuela-Ríos 1999). The Devonian period features Palmatolepis triangularis, an important guide fossil for the Lower Devonian Emsian stage (Ziegler and Sandberg 1990), and Icriodus woschmidti, which helps define the Lower-Middle Devonian boundary (Klapper 1988).

For the Carboniferous period, various Idiognathodus species, such as *Idiognathodus simulator* and *Idiognathodus sinuatus*, serve as significant index fossils in the United States and Europe (Heckel 2008). In the Permian period, Neostreptognathodus pnevi is used to define the Permian-Triassic boundary in the Urals and Siberia (Chernykh and Ritter1997), while Sweetognathus whitei is an important guide fossil for the uppermost Permian strata in the Western United States (Clark and Carr 1984).

During the Triassic period, Hindeodus parvus serves as an index fossil for the Lower-Middle Triassic boundary (Kozur 2003), and Isarcicella isarcica helps determine the Anisian-Ladinian boundary in various parts of the world (Orchard 2007). These conodont species provided valuable insights into the geological history and evolution of ancient marine environments

Environmental Impact on Conodont Evolution

The evolution of conodonts was significantly influenced by environmental changes. These changes included fluctuations in sea level, ocean temperature, and global climate, all of which played crucial roles in shaping the diversity and distribution of conodont species over millions of years.

Sea-level changes: Changes in sea level had a profound impact on conodont evolution. Sea-level fluctuations, driven by tectonic activities and glacial cycles, altered marine habitats and affected the distribution and diversification of conodonts. During periods of sea-level rise (transgressions), shallow marine environments expanded, providing new habitats for conodonts. Conversely, sea-level falls (regressions) reduced shallow marine areas, leading to habitat loss and sometimes extinction of conodont species (Sweet 1988). These cycles created dynamic environments that drove evolutionary adaptation and speciation. Sea-level changes often influenced oceanic circulation patterns, leading to anoxic (oxygen-depleted) events in certain marine basins. Anoxic conditions, which are detrimental to most marine life, resulted in significant turnover in conodont populations. Certain conodont species adapted to low-oxygen conditions, while others went extinct, illustrating the impact of environmental stress on their evolution (Donoghue and Purnell 1999).

Ocean temperature and climate: The global climate and ocean temperatures played a critical role in conodont evolution. Variations in climate, often associated with glacial and interglacial periods, influenced marine ecosystems and the distribution of conodont species. Conodont elements have been used to reconstruct past ocean temperatures, providing insights into the environmental conditions that influenced their evolution. The color alteration index (CAI) of conodont fossils, which changes with thermal maturity, serves as a paleotemperature proxy (Purnell et al., 2000). These reconstructions show that conodont diversity was higher in warmer periods and lower during cooler periods.

Major climate shifts, such as those occurring during the Ordovician-Silurian transition and the Late Devonian extinction, had significant impacts on conodont populations. Cooling events and associated environmental changes led to mass extinctions, including the disappearance of many conodont species. Conversely, warmer periods fostered the diversification and proliferation of conodonts (Aldridge et al., 1986). Conodonts became extinct in Late Triassic (Sweet 1988).

Ecological niches and adaptations: The evolution of conodonts was influenced by the ecological niches they occupied, adapting to a variety of marine environments from shallow, warm waters to deeper, cooler regions.

Morphological adaptation: The diversity of conodont element shapes and structures reflects their adaptation to different feeding strategies and ecological roles. For instance, variations in conodont apparatuses suggest adaptations to different prey types and feeding mechanisms, driven by the availability of resources in their environments (Donoghue and Purnell 1999).

Morphological characteristics: Morphological characteristics of conodonts can be categorized into several distinct types, each showcasing unique features that aid in their identification and classification. According to Müller (1979), these categories include (Table 1) different morphological types.

Among these, platform conodonts-which evolved from blade types are particularly significant. These include *Palmatolepis hassi* from the Upper Devonian in Iowa, *Neogondolella prava* from the Middle Triassic in Germany, *Polygnathoides* from the Middle Silurian, and *Kockellela* from the Late Silurian. These species highlight the diversity and complexity of conodonts, underscoring their importance in geological studies. Figure 1 displays images of conodonts representing various morphotypes, including their generic and species names, as well as the formation and site of origin.

Habitat specialization: Some conodont species became highly specialized, adapting to specific environmental

Table 1. Conodont morphological types and species

Morphological type	Characteristics	Notable Species	Geological period/Location
Simple cones	Single tooth-shaped denticles	*Furnishina furnishi*	Upper Cambrian, Sweden
		Scolopodus rex	Lower Ordovician, Germany
		Ulrichodina	Ordovician
Bar types	Thin, curved, or bent shafts	*Ligonodina*	Silurian, Germany
		Hibbardella	Upper Silurian, Germany
Blade types	Elongate, laterally compressed with fused denticles	*Pterospathodus amorphognathoides*	Upper Silurian, Austria
		Ozarkodina immersa	Upper Devonian, Michigan
Platform conodonts	Derived from blade types	*Palmatolepis hassi*	Upper Devonian, Iowa
		Neogondolella prava	Middle Triassic, Germany
		Polygnathoides	Middle Silurian
		Kockellela	Late Silurian

conditions such as reef environments or deep-water settings. This specialization often led to greater vulnerability to environmental changes, contributing to periodic extinctions when conditions shifted (Purnell et al., 2000).

Mass extinctions and environmental stress: Conodonts thrived from the Cambrian period until their extinction at the end of the Triassic period, around 201.3 million years ago. The precise timing of the conodont extinction aligns with the end-Triassic mass extinction event, which was one of the five largest extinction events in Earth's history. This event marked a significant ecological shift and is believed to have been caused by massive volcanic eruptions from the Central Atlantic Magmatic Province (CAMP), leading to dramatic climate changes, ocean acidification, and widespread habitat loss (Racki 1999, Benton 2003).

Several mass extinction events throughout the Paleozoic era had profound effects on conodont evolution. These events were often triggered by dramatic environmental changes, such as volcanic activity, asteroid impacts, and significant shifts in climate. The Ordovician-Silurian extinction, caused by a combination of glaciation and sea-level fall, led to the extinction of many marine species, including numerous conodonts. The recovery and subsequent radiation of conodonts in the Silurian period illustrate their resilience and ability to adapt to new environmental conditions (Sweet 1988).

Similarly, the Late Devonian extinction, marked by a series of environmental disturbances including anoxic events and global cooling, had a significant impact on conodont diversity. Many species went extinct, but the event also paved the way for the emergence of new conodont lineages adapted to the altered environments (Aldridge et al., 1986). Despite these catastrophic events, conodonts continued to thrive until the end-Triassic mass extinction, underscoring their resilience and adaptability in the face of environmental stress.

Fig. 1. Images of conodonts providing a general overview of different morphotypes (Adopted from https://www.ucl.ac.uk/GeolSci/micropal/conodont.html)

The Rhaetian age is the latest age of the Triassic period, immediately preceding the Jurassic period. It spans from approximately 208.5 to 201.3 million years ago. The Rhaetian age marks the final stage of the Triassic, leading up to the Triassic-Jurassic extinction event, which was one of the major mass extinction events in Earth's history (Gradstein et al., 2012, Cohen, et al., 2013)

Biogeographical distribution of conodonts: Conodonts exhibit a wide geographical distribution that spans several geological periods from the Cambrian to the Triassic. Their distribution patterns provide valuable insights into past marine environments, plate tectonics, and biogeographic provinces.

Conodonts have been discovered on every continent, reflecting their widespread presence in ancient marine environments. Their fossils are found in a variety of sedimentary rocks, indicating their adaptability to different marine conditions. The earliest conodonts appear in the Cambrian strata of North America, Europe, Asia, and Australia, providing critical information about the early diversification of conodonts and their initial spread across the world's oceans (Miller 1984). During the Ordovician to Silurian periods, conodonts reached a high degree of diversity and widespread distribution. Significant conodont assemblages from these times have been reported from regions such as the Appalachian Basin in North America, the Baltic region, China, and the Australasian region, indicating extensive shallow marine habitats across these regions (Sweet 1988).

The Devonian period is marked by extensive reef-building and diverse conodont faunas, with major conodont-bearing formations found in North America, Europe, South America, and Australia. The presence of conodonts in reef environments highlights their role in the complex marine ecosystems of the Devonian seas (Aldridge et al., 1986). Conodonts continued to be widely distributed during the Carboniferous and Permian periods, with significant assemblages found in North America, Europe, Russia, China, and Australia. These periods saw the development of distinct conodont biogeographic provinces, influenced by climatic zones and ocean circulation patterns (Clark et al., 1981). Conodonts persisted into the Triassic period before their eventual extinction in the late Triassic, with Triassic conodonts found in Europe, North America, and Asia, indicating their continued wide distribution in marine environments until their extinction (Mertmann 2003).

The distribution of conodonts is often divided into distinct biogeographic provinces, reflecting the influence of paleogeography, climate, and ocean currents on their distribution. During the Paleozoic era, tropical and subtropical conodont provinces were characterized by high diversity and endemism. These provinces include the Laurentian province (North America), the Baltic province (Northern Europe), and the South China province, where conodont faunas were adapted to warm, shallow marine environments (Sweet 1988). Conodonts from temperate and boreal regions exhibit different assemblages compared to tropical provinces. These provinces, such as the Siberian province and the Australasian province, were characterized by cooler water faunas with distinct species adapted to temperate conditions (Clark et al., 1981). Conodonts are also found in deep-water deposits, indicating their adaptability to different marine settings. Deep-water conodont faunas are typically less diverse but provide important information about the paleoenvironments of ancient ocean basins (Miller 1984).

Plate tectonics and biogeographical distribution of conodonts: Plate tectonics played a significant role in shaping the geographical distribution of conodonts. The movement of tectonic plates influenced ocean currents, climate, and the formation of marine basins, all of which affected conodont habitats. The distribution of conodonts is used to reconstruct paleogeographic maps of ancient Earth. By analyzing conodont faunas from different regions, geologists can infer the positions of continents and the configuration of ancient oceans (Scotese 2001). Tectonic events such as the uplift of mountain ranges and the subsidence of basins created new marine environments that conodonts colonized, contributing to the dynamic distribution patterns observed in the conodont fossil record (Miller 1984). Changes in ocean circulation, driven by plate movements, affected the distribution of conodonts by influencing sea temperature, salinity, and nutrient availability. These factors played a critical role in the biogeographic differentiation of conodont faunas (Aldridge et al., 1986).

The study of conodonts, which are extinct microfossils of jawless vertebrates, has provided significant insights into the geological history of Earth, including the mechanisms of plate tectonics. The distribution, evolution, and extinction of conodonts are closely tied to the tectonic activities that have shaped our planet's continents and oceans. Conodonts lived in marine environments, and their fossilized remains have been found in sedimentary rocks across the world. The movement of tectonic plates over geological time has played a crucial role in the distribution of these fossils. During the Paleozoic era, the continents were arranged differently than they are today. The breakup of supercontinents and the subsequent formation of new ocean basins influenced the habitats and distribution of marine organisms, including conodonts (Sweet 1988).

During the Cambrian to Ordovician periods, the supercontinent Gondwana was breaking apart, and the opening of new ocean basins provided extensive shallow marine environments suitable for conodonts. The distribution of conodont fossils from this time shows their adaptation to these changing marine environments, reflecting the influence of plate tectonics on their habitats (Donoghue and Purnell 1999). The formation of the supercontinent Euramerica (Laurussia) and the subsequent closing of the Iapetus Ocean during the Silurian to Devonian periods had significant effects on marine biodiversity. Conodonts from these periods exhibit significant evolutionary changes, likely driven by the changing marine environments and ecological niches resulting from tectonic activity (Purnell et al., 2000). The assembly of the supercontinent Pangaea during the late Paleozoic era brought together previously separated marine environments, leading to increased competition and ecological pressures. The distribution of conodonts during this time reflects the merging of different faunal provinces and the impact of tectonic collisions on marine ecosystems (Aldridge et al., 1986). The breakup of Pangaea began in the Triassic period, leading to the formation of the Atlantic Ocean. This tectonic activity influenced the distribution of marine environments and the eventual decline and extinction of conodonts. The changing sea levels and the creation of new oceanic gateways affected marine circulation patterns, impacting conodont populations (Sweet 1988).

Conodonts have been used as bio-stratigraphic markers to date and correlate sedimentary rock layers, providing valuable information about past tectonic events. Their rapid evolutionary rates and widespread distribution make them ideal for this purpose. Conodonts have been used to date and understand the timing of orogenic (mountain-building) events. For instance, the presence of certain conodont species in deformed sedimentary rocks can indicate the timing of tectonic uplift and deformation (Donoghue and Purnell 1999). Plate tectonics also influences global sea levels. The transgressive and regressive sequences recorded in sedimentary rocks often contain conodont fossils, which can be used to correlate these sequences globally and understand the relationship between tectonic activity and sea-level changes (Aldridge et al., 1986). The distribution of conodont species across different continents has helped in reconstructing past continental configurations and the movement of tectonic plates. By comparing conodont faunas from different regions, geologists can infer the relative positions of continents and the opening and closing of oceanic gateways (Purnell et al., 2000).

Significance of Conodont Color Alteration Index (CIA) values: This review elucidates the pivotal role of the Conodont Color Alteration Index (CAI) in geology, highlighting its importance in understanding the thermal history of rocks and tectonic processes. The CAI, a numerical scale quantifying the color changes in conodont elements due to thermal alteration, provides valuable insights into geological processes (Epstein et al., 1977). Conodonts, composed of calcium phosphate, exhibit predictable color changes with increasing temperature, allowing researchers to correlate these changes with specific temperature ranges (Aldridge et al., 1993). Tectonic activities such as burial, metamorphism, and uplift influence the CAI, enabling the inference of geological processes' intensity and duration. Low CAI values suggest minimal thermal alteration, indicative of shallow burial or recent uplift, while higher CAI values imply deeper burial and prolonged exposure to elevated temperatures (Königshof 2003). Different tectonic settings result in distinct thermal histories, with subduction zones and collisional mountain-building events leading to high-temperature metamorphism (Rejebian et al., 1987). In stratigraphy, CAI values assess the thermal maturity of sedimentary basins, revealing changes in burial history related to tectonic events (Harris 2007). Additionally, CAI values estimate paleogeothermal gradients, aiding in reconstructing the thermal evolution of basins. Despite its semi-quantitative nature and the need for careful interpretation considering local conditions, the CAI remains a valuable tool for unraveling the tectonic history recorded in sedimentary rocks (Merrill 1991). CAI values range from unaltered (light gray to greenish gray) to extremely altered (black or very dark gray), with specific colors varying by researcher and rock type, necessitating their use alongside other geological data for comprehensive analysis (Fig. 2).

Importance of conodonts in determining paleoclimate: Conodonts have been instrumental in paleoclimatology—the study of past climates. The detailed fossil record of conodonts, along with the chemical and physical properties of their remains, provides critical insights into the Earth's climatic history.

One of the primary methods by which conodonts contribute to paleoclimate studies is through the Conodont Color Alteration Index (CAI). The CAI measures the degree of thermal alteration of conodont elements, which changes their color in a predictable manner with increasing temperature. The CAI is widely used to determine the thermal maturity of sedimentary rocks, which is essential for understanding the thermal history of a region. By correlating CAI values with known geological events and rock temperatures, scientists can reconstruct past geothermal gradients and heat flow, providing indirect evidence of paleoclimatic conditions (Epstein et al., 1977). Changes in the Earth's geothermal gradient over time are often linked to broader climatic changes. For instance, variations in CAI values across different stratigraphic layers can indicate periods of increased volcanic activity or tectonic movements, which in turn affect global climate patterns (Donoghue and Purnell 1999).

Isotopic analysis of conodont elements, particularly oxygen isotopes, offers direct insights into past ocean temperatures and, by extension, climate conditions. The ratio of oxygen isotopes (^18O/^16O) in conodont apatite is a robust indicator of the temperature of the seawater in which the conodonts lived. Higher ratios generally indicate cooler water temperatures, while lower ratios suggest warmer conditions. This method has been used to reconstruct detailed temperature records for various geological periods, providing evidence of climatic fluctuations such as ice ages and interglacial periods (Grossman 2012). Conodont oxygen isotope ratios serve as proxies for ancient ocean temperatures, allowing scientists to chart long-term climate trends and correlate them with other paleoclimatic data such as ice core records and sediment cores. This helps in understanding the drivers behind historical climate changes and their impact on marine life (Shields et al., 2003).

The distribution and diversity of conodont species in the fossil record reflect past environmental conditions, offering indirect evidence of paleoclimate. The presence and abundance of specific conodont species in different regions can be correlated with past climatic conditions. For example, certain conodont species thrived in warm, shallow seas, while others were adapted to cooler, deeper waters. Analyzing changes in their distribution patterns helps reconstruct past climate zones and marine environments (Purnell et al., 2000). Major climatic events, such as global cooling or warming, often correspond with significant changes in conodont diversity. Periods of rapid climate change are frequently marked by conodont extinctions, followed by the radiation of new species adapted to the altered conditions. These patterns provide insights into how climate change impacts biodiversity and ecosystem dynamics (Sweet 1988).

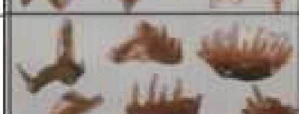
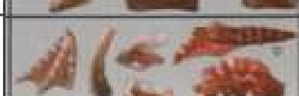
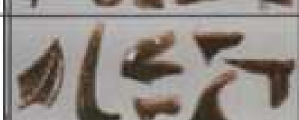
Colour Alteration Index	Colour Alteration	Temperature Range (degree)
1		<15-80
1.5		50-90
2		60-140
3		110-200
4		190-300
5		+300

Fig. 2. Colour Alteration Index of Conodonts (After Epstein et al., 1977 and Harris et al., 1978)

Conodont CAI values and oxygen isotope ratios have been used to study the Ordovician-Silurian ice age, revealing significant cooling events and their impact on marine ecosystems. The data indicate a correlation between glaciation events and conodont extinction and radiation patterns (Melchin et al., 2013). Isotopic analyses of conodonts from the Late Devonian period have provided evidence of global cooling and anoxia, which contributed to one of the major mass extinctions in Earth's history. These studies help understand the interplay between climate change and biological crises (Joachimski et al., 2009).

Economic importance of conodonts: Conodonts have significant economic importance primarily due to their use in the fields of petroleum geology and stratigraphy. Their fossilized remains, especially the tooth-like elements, have proven invaluable in various geological applications. Conodonts are crucial to oil exploration due to their utility in biostratigraphy, thermal maturity assessments, and paleoenvironmental reconstructions. These applications help geologists identify potential hydrocarbon reservoirs, evaluate the thermal maturity of source rocks, and reconstruct past depositional environments, thereby guiding exploration efforts including mineral exploration.

Petroleum exploration: Conodonts are critical in biostratigraphy. Their widespread distribution, rapid evolutionary changes, and distinct morphological features make them excellent bio-stratigraphic markers. The presence of specific conodont species in sedimentary rock layers allows geologists to accurately date these layers and correlate them across different geographic regions. Understanding these sequences allows geologists to predict the location of reservoir rocks, source rocks, and seals within a basin (Kauffman and Ziegler 1997). This is particularly useful in the oil and gas industry, where understanding the age and distribution of rock formations is crucial for exploration and extraction activities (Sweet 1988). Conodont biostratigraphy helps in identifying and characterizing source rocks, which are the sedimentary rocks from which hydrocarbons are generated. This approach helps in understanding the depositional history and sedimentary environment of rock formations, which is essential for predicting the location of reservoirs, source rocks, and seals in hydrocarbon systems (Aldridge et al., 1986). By determining the age and depositional environment of these rocks, geologists can assess their potential for hydrocarbon generation and identify the most promising exploration targets (Donoghue and Purnell 1999). Conodont assemblages provide insights into past ecological conditions and sea-level changes. By studying the distribution and diversity of conodonts in different stratigraphic layers, geologists can reconstruct past marine environments and identify transgressive-regressive cycles, which are important for understanding sediment deposition and reservoir quality (Sweet 1988).

By reconstructing past marine environment, geologists can identify the types of sediments and structures that are likely to host hydrocarbons (Purnell et al., 2000). Additionally, conodont oxygen isotope analysis provides insights into past ocean temperatures, helping to reconstruct paleoclimate conditions. This information is critical for understanding the thermal history of sedimentary basins and for predicting the quality and distribution of potential reservoir rocks (Grossman 2012).

The Conodont Color Alteration Index (CAI) is a key tool for assessing the thermal maturity of sedimentary rocks, critical for evaluating their potential to generate hydrocarbons. The CAI measures the extent of thermal alteration in conodont elements, which correlates with the temperature history of the host rock. CAI values range from 1 (indicating low temperatures) to 6 (indicating high temperatures). By determining the CAI values of conodonts in a rock sequence, geologists can assess whether the rocks have reached the temperatures necessary for hydrocarbon generation (Epstein et al., 1977). Rocks must be within a specific temperature range, known as the oil window (60-120°C), to generate oil. CAI provides a direct indication of whether a rock unit has been subjected to sufficient heat to generate hydrocarbons, aiding in the identification of prospective source rocks (Donoghue and Purnell 1999). In developed oil fields, conodont biostratigraphy is used to refine reservoir models and improve the understanding of the spatial distribution of oil-bearing strata, leading to more efficient extraction strategies and enhanced recovery rates (Kauffman and Ziegler, 1997).

Practical application in oil exploration: Case history of Kutch (Kachchh) oil exploration: India has a rich geological history, with several sedimentary basins that are of interest for hydrocarbon exploration. Conodont biostratigraphy has been effectively utilized in various Indian sedimentary basins to aid in oil exploration. One prominent case is the use of conodonts in the Kutch Basin, located in the western part of India.

The Kutch Basin is a Mesozoic to Cenozoic sedimentary basin situated in the state of Gujarat, western India. It is known for its thick sequences of marine and continental sediments, which have been the focus of extensive geological studies due to their potential for hydrocarbon reserves (Biswas 1982). Conodonts have been employed to establish a detailed bio-stratigraphic framework for the Kutch Basin. The presence of distinct conodont species has allowed geologists to date the sedimentary sequences accurately. This precise dating is crucial for correlating rock layers within the basin and with other basins, thereby facilitating the identification of potential hydrocarbon-bearing strata (Jauhri and Agarwal 2001).

The Conodont Color Alteration Index (CAI) has been used to assess the thermal maturity of the sedimentary rocks in the Kutch Basin. By determining the CAI values of conodont elements, geologists have been able to evaluate the thermal history of the basin. This assessment helps in identifying whether the rocks have been subjected to temperatures sufficient for hydrocarbon generation (Gupta 1999). Conodonts, along with other microfossils, have been used to reconstruct the paleoenvironmental conditions of the Kutch Basin. This reconstruction helps in understanding the depositional environments, which is critical for predicting the presence and quality of reservoir rocks. The data obtained from conodont studies indicate that the Kutch Basin experienced significant marine transgressions and regressions, influencing the distribution of potential reservoir rocks (Singh et al., 2012).

The bio-stratigraphic data derived from conodont studies in the Kutch Basin have been used to correlate the sedimentary sequences with those in other basins in India and globally. These correlations provide a broader context for understanding the geological history and potential hydrocarbon systems of the region (Jauhri and Agarwal 2001). Studies have identified well-preserved conodonts from the Middle to Late Triassic sequences in the Kutch Basin. The identification of these conodonts has provided a precise age framework for these strata, which are considered potential source rocks for hydrocarbons. The CAI values indicate that these rocks have undergone appropriate thermal maturation for oil generation (Gupta 1999).

Oxygen isotope analyses of conodont elements from the

Kutch Basin have provided paleotemperature estimates for the ancient marine environments. These estimates help in understanding the thermal gradients and conditions favorable for hydrocarbon formation and preservation (Singh et al., 2012). The integration of conodont biostratigraphy with other geological and geophysical data has led to successful identification and development of hydrocarbon prospects in the Kutch Basin. The precise dating and thermal maturity assessments provided by conodont studies have reduced exploration risks and guided drilling decisions (Jauhri and Agarwal 2001).

The use of conodonts in the Kutch Basin exemplifies their value in oil exploration. Through bio-stratigraphic zoning, thermal maturity assessments, paleoenvironmental reconstructions, and basin correlations, conodonts have significantly contributed to understanding the geological framework and hydrocarbon potential of this important sedimentary basin.

Mineral Exploration

Conodonts also play a role in mineral exploration. Their presence in certain sedimentary rocks can help identify and characterize mineral deposits. Conodont elements are composed of apatite, a phosphate mineral. Their accumulation in sedimentary rocks can indicate the presence of phosphorite deposits, which are economically important as a source of phosphorus for fertilizers and other industrial applications (Donoghue and Purnell 1999). Conodonts can be found in metalliferous shales, which are sedimentary rocks rich in metals such as lead, zinc, and silver. These shales often form in specific depositional environments that can be identified through the study of conodont assemblages, aiding in the exploration and exploitation of metal resources (Aldridge et al., 1986).

RESULTS AND DISCUSSION

The earliest conodont elements, appearing during the Cambrian period, are simple cone-shaped structures categorized within the Protoconodontida and Paraconodontida groups (Sweet 1988). These early forms suggest that conodonts were among the first vertebrates to develop hard tissues, marking a significant evolutionary milestone. During the Ordovician period, conodonts diversified rapidly, giving rise to complex apparatuses with various element types such as pectiniform and ramiform, indicating changes in feeding strategies and ecological adaptations (Donoghue and Purnell 1999). The peak diversity of conodonts during the Silurian and Devonian periods is characterized by the development of multi-element apparatuses and adaptations to various marine environments, reflecting the diversification of marine ecosystems at that time (Purnell et al., 2000).

The decline in conodont diversity during the Triassic period and their eventual extinction by the Late Triassic is likely linked to dramatic environmental changes and mass extinctions occurring at that time (Sweet 1988). This decline highlights the sensitivity of conodonts to environmental stresses and the impact of global changes on marine biodiversity.

The anatomical study of conodonts has provided significant insights into their functional morphology and feeding mechanisms. Early conodonts had relatively simple feeding structures, but over time, they evolved complex apparatuses consisting of multiple element types, each likely serving different functions within the conodont's mouth, such as grasping, slicing, and grinding food (Donoghue and Purnell 1999). The discovery of well-preserved conodont fossils revealed that they were soft-bodied, eel-like creatures with large eyes and a notochord. This anatomical arrangement suggests that conodonts used a complex feeding apparatus, similar in some respects to the teeth of modern vertebrates (Aldridge et al., 1986).

Conodonts are invaluable in biostratigraphy due to their rapid evolutionary rates and distinct morphological changes over time. Their widespread distribution allows for the correlation of rock layers over vast geographic areas, making them ideal for dating and correlating sedimentary sequences (Sweet 1988, Donoghue and Purnell 1999). Conodont biozones are used worldwide to delineate geological time periods, particularly in the Paleozoic era, providing a high-resolution tool for geological studies (Purnell et al., 2000).

The evolution of conodonts was significantly influenced by environmental changes, including fluctuations in sea level, ocean temperature, and global climate. Sea-level changes, driven by tectonic activities and glacial cycles, altered marine habitats and affected the distribution and diversification of conodonts. Periods of sea-level rise provided new habitats, while sea-level falls led to habitat loss and sometimes extinction of conodont species (Sweet 1988). Ocean temperature and climate also played critical roles in conodont evolution, with higher diversity observed during warmer periods and lower diversity during cooler periods (Aldridge et al., 1986).

Conodonts are often found in association with various other fossil groups, providing valuable insights into paleoecology and depositional environments. Their co-occurrence with brachiopods, trilobites, corals, bryozoans, ammonoids, nautiloids, radiolarians, and foraminifera helps in reconstructing ancient marine environments and aids in dating and correlating marine sedimentary rocks across different regions (Miller 1984, Aldridge et al., 1986, Sweet1988, Clark et al., 1981).

Plate tectonics played a significant role in shaping the geographical distribution of conodonts. The movement of tectonic plates influenced ocean currents, climate, and the formation of marine basins, affecting conodont habitats. The distribution of conodonts is used to reconstruct paleogeographic maps of ancient Earth, providing insights into past marine environments and the positions of continents (Scotese 2001). Tectonic events such as the uplift of mountain ranges and the subsidence of basins created new marine environments that conodonts colonized, contributing to the dynamic distribution patterns observed in the conodont fossil record (Miller 1984).

Conodonts have been instrumental in paleoclimatology and economic geology. The Conodont Color Alteration Index (CAI) and isotopic analysis of conodont elements provide insights into past ocean temperatures and climatic conditions (Epstein et al., 1977, Grossman 2012). Additionally, conodonts are crucial in oil exploration due to their use in biostratigraphy, thermal maturity assessments, and paleoenvironmental reconstructions, guiding exploration efforts and refining reservoir models (Kauffman and Ziegler1997).

CONCLUSION

The study of conodonts provides profound insights into the evolutionary history of early vertebrates, environmental adaptations, and Earth's geological past. Their extensive fossil record and diverse adaptations reflect significant evolutionary responses to environmental changes and biogeographical shifts influenced by plate tectonics. Serving as index fossils, conodonts are indispensable in biostratigraphy and petroleum geology, facilitating the dating and correlation of sedimentary rocks and guiding hydrocarbon exploration. Moreover, their role in reconstructing paleoclimates underscores their importance in understanding historical climate dynamics and their impact on marine life. Conodonts represent a critical window into ancient marine ecosystems and the evolutionary history of life on Earth. Conodonts have significant economic importance primarily due to their use in the oil exploration and in the search of phosphatic mineral deposits.

While this review provides a comprehensive overview of conodonts' evolutionary, bio-stratigraphic, environmental, and economic significance, it is essential to acknowledge some limitations. The review relies on available published literature, which may introduce biases or overlook recent findings not yet incorporated into the scientific discourse. Additionally, interpretation of conodont fossils and their implications may vary among researchers, leading to differing conclusions. Furthermore, the scope of this paper may not encompass every aspect of conodont research, leaving room for further exploration and inquiry into this fascinating group of organisms.

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Conservation Priority of Indian Tropical Dry Deciduous Habitat: Case Study in Panna Based on Potential Regeneration of Tree Species

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Abstract: The Panna Tiger Reserve's conservation efforts serve as an excellent example of sustainable wildlife conservation. Therefore, to better understand the features of tropical dry deciduous forests of PTR, various metrics were used, such as species density and regeneration status of different tree species. The findings indicate that 19 tree species were designated as good regenerators, while 36.956 percent of tree species were deemed non-regenerative . PCA and Multiple Linear Regression (MLR) studies were utilized to find the relationship between the different life forms of plants and the predictor or environmental variables. The PCA-MLR Model predicted tree, seedling, and sapling density accurately and revealed that shrub density, shrub cover, and disturbance variables such as lopping, cutting, cattle dung, and grazing were the most important contributors to lowering tree density. In addition, shrub density, shrub cover, and cattle dung were the most important elements influencing seedling density, whereas herb density, grass density, tree cover (%), and height were the most important factors influencing sapling density.

Keywords: Principal component analysis, Panna Tiger Reserve, Tropical dry deciduous forest, Regeneration

The tropical forest is a remarkable and diverse environment, both ecologically and genetically, covering almost half of all forest areas. It offers a range of environmental benefits and supporting the livelihoods of millions of people worldwide (Cunningham et al., 2008, Waeber et al., 2012). However, these forests are increasingly facing anthropogenic disturbances that threaten their survival. Unsustainable practices such as farmland conversion, over-harvesting of valuable wood species, and the burning of forests have led to habitat fragmentation, forest depletion, and changes in rejuvenation patterns (Menaut et al., 1995, Saha and Howe 2003). This degradation of the world's dry forests has severe implications for the environment, society, and economy. Forests exhibit notable phenological adaptations, such as distinct leaf features ranging from evergreen to deciduous leaf habits (Sandquist and Cordell 2007). In India, tropical dry forests cover a significant portion, up to 60%, of the country's forested land (Waeber et al., 2012). Among these, tropical dry deciduous forests represent approximately 40% of the total tropical dry forest area (ISFR 2019). This underscores the ecological importance of tropical dry forests in the Indian subcontinent. Numerous studies have highlighted the deteriorating condition of these ecosystems, emphasizing the need for urgent conservation efforts. (García-Cervigón 2017, Lindenmayer et al., 2017, Sambe et al., (2018), Temperton et al., 2019, Etter et al., 2020, Pratzer2021, Buchadas et al., 2022, Liu et al., 2022). The study aimed to provide a basic understanding of species density and the interaction between the environment and the forest community of the Panna tiger reserve.

MATERIAL AND METHODS

Study area: The Panna Tiger Reserve in northern Madhya Pradesh's Vindhaya Hill is a more delicate environment for Tigers. It is located in the districts of Panna and Chattarpur in Madhya Pradesh. It is controlled as the core area and covers an area of roughly 576.12 square kilometers. The Tiger Reserve has six forest typessouthern tropical dry deciduous teak forest, northern tropical dry deciduous mixed forest, dry deciduous scrub forest, *Anogeissus pendula* forest, *Boswellia* forest, and dry bamboo brakes. This forest is dominated by a broad plateau and gorges. The Ken River, which has become a lifeline for the reserve and also generates waterfalls on its route to the valley, distinguishes the woodland (Fig. 1).

Data collection: A stratified random sampling approach was implemented for conducting extensive vegetation sampling in the area. This method involved the use of the lowest administrative unit, the forest beat, for stratification purposes. A total of 34 line transects were established, covering the entire area of the tiger reserve's 34 beats. The survey of 338 plots provided comprehensive coverage of 10.6132 hectares of forest land.The transect, which was two kilometers long and segmented into 200 parts, was used at each sample plot. A circular plot with a radius of 10 meters was used, which contained nested 5-meter-radius plots. The density of tree species was determined using a 10-meter-radius circular plot, while a nested 5-meter-radius circular plot was employed to estimate the number of shrubs, seedlings and saplings. The density of grasses and herbs was measured in four 0.5x0.5m quadrants at four random places inside a 10m circular plot.

The tree cover was measured using a gridded mirror, while the shrub cover was estimated visually. Ground cover, including herb cover (%), grass cover (%), bare ground (%), withered stone (%), litter (%), and rock (%), was recorded using the Point-intercept method. Additionally, signs of human activity, such as animal grazing, were noted within a 10 m radius of each sampling plot. Geographic information system (GIS) tools were used to determine the distance to the nearest water body, forest road, and human habitation. Topographic features like altitude, slope, and aspect were obtained using a digital elevation model.

Data Analysis

Density: The number of the tree species and their regenerating species (seedling and sapling) was estimated as "number of individuals/ ha".

Density = number of individuals of the species / area (ha)

Regeneration status: The forest's regeneration was measured according to Shankar (2001) and Singh et al. (2017) using the following criteria.

I. Good if the density of Seedlings> Saplings>Trees

II. Fair if the density of Seedling>Sapling<Trees

III. Poor if the species present only in the sapling stage but not in the seedling stage.

IV. Not regenerating if the species is absent in both seedling and sapling stages but only found as an adult tree

Canonical correspondence analysis (CCA): The study used CCA direct ordination to analyze the corelation and regression between floristic data and habitat variables (Ter Braak and Prentice 1988, Kent and Coker 1992).

Principal component analysis (PCA): The original variables are weighted linear combinations of the principal components. The Kaiser-Meyer Olkin (KMO) and Bartlett's test measures of sample adequacy were utilized to validate the applicability of PCA to the data. VARIMAX rotation approach was also used to maximize the difference in factor loading on each principal component (Terdalkar and Pai 2001).

Multiple linear regression (MLR): To identify the best predictors of plant communities, conducted multiple linear regression analysis of various plant life forms, such as trees, seedlings, and saplings, using stepwise variable selection processes on the principal component scores.

Statistical analysis: The square root and arcsine transformations were applied before conducting multivariate analysis. SPSS version 7.5 and Past 3.1 software (version 3.1, Øyvind Hammer, Natural History Museum, University of Oslo) were used for all analyses.

RESULTS AND DISCUSSION

Canonical correspondence analysis (CCA): The direct gradient analysis using CCA was employed to examine the relationship between environmental factors and tree species composition in five distinct habitats (Fig. 2). Monte Carlo

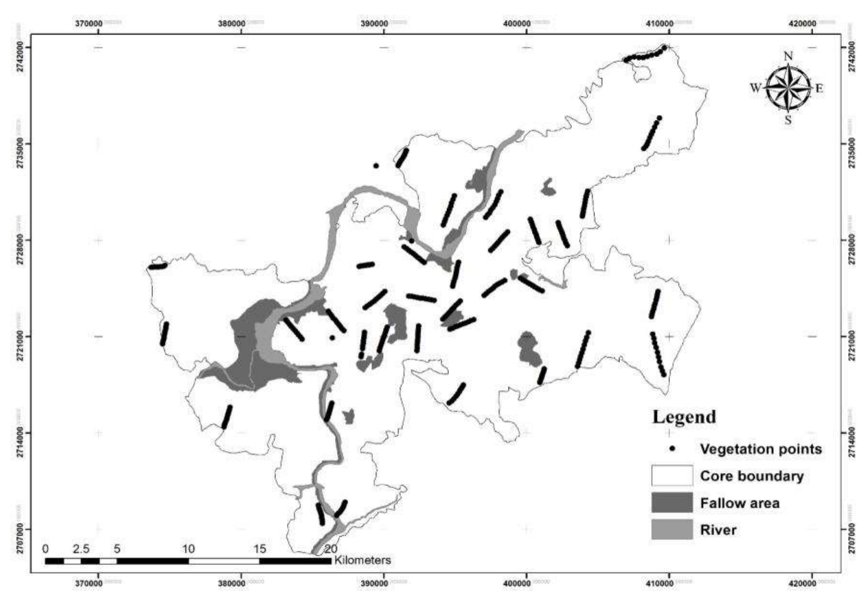

Fig. 1. Vegetation points along the transects

permutation test (100 iterations) indicated a lack of significant correlation between species abundance and the supplied environmental variables. The four axes (eigenvalue, axis 1= 0.433, axis 2= 0.169, axis 3= 0.091 and axis 4= 0.048) of the CCA ordination analysis explained 58.35, 22.84, 12.3, and 6.50% of the variance in tree species assemblages with the predictive variables (Table 2). In the mixed-thorn habitat, the species *Zizyphus xylopyra*, *Phyllanthus emblica*, and *Pterocarpus marsupium* exhibited a strong correlation with positive scores on axis 4, while *Tectonagrandis* demonstrated a moderate correlation with a positive score on axis 4. The proximity to a water body and disturbance factors such as tree cutting and cattle dung emerged as significant predictors for these tree species.

In the habitat characterized by a mix of thorny vegetation, the plant species *Zizyphus xylopyra*, *Phyllanthus emblica*, and *Pterocarpus marsupium* displayed a noteworthy strong correlation with positive scores on axis 4. Similarly, *Tectona grandis* demonstrated a moderate correlation with a positive score on axis 4. Moreover, the proximity to a water body and the presence of disturbance factors such as tree cutting and cattle dung were identified as influential predictors for the distribution of these tree species. The species *Butea monosperma* and *Acacia catechu* were observed in the lower right quadrant. These species strongly correlated with the Mixed-thorn habitat on the second axis. This correlation was predicted by lower tree height, tree GBH (Girth at Breast Height), shrub cover percentage, and tree cover percentage (Table 2, Fig. 2).

The positive scores on axis 2, specifically in the upper left quadrant, predominantly comprised *Wrightia tinctoria*, *Lagerstroemia parviflora*, and *Boswellia serrata*. In the mixed habitat type, other significant disturbance gradients included the lopping of trees, grazing, percentage of grass cover, percentage of herb cover, as well as grass and herb density. The teak-mixed habitat in the lower left included a stunning array of trees such as *Buchanania lanzan*, *Ceriscoides turgida*, *Scheichera trijuga*, *Balanites aegyptiaca*, *Diospyros melanoxylon*, *Terminalia bellirica*, *Limonia acidissima*, *Careya arborea*, and *Adina cordifolia*. These trees showed a positive correlation with shrub density and cover percentage in the teak-mixed habitat on axis 3. However, *Holoptelea integrifolia*, *Abrus precatorius*, *Albizzia procera*, and *Bombax ceiba* exhibited a negative correlation with both shrub density and shrub cover in the teak-mixed habitat. *Aegle marmelos* and *Terminalia bellirica* were situated in the lower right axis. These species exhibited a stronger correlation with the teak-mixed habitat along axis 3, while demonstrating a negative association with tree GBH, tree density and tree cover (%).

In the mixed with dense understory, certain tree species such as *Stephegyne parvifolia*, *Bassia latifolia*, and *Bauhinia racemose* exhibited a negative correlation with tree cover (%), shrub cover (%), higher tree density, tree height, tree GBH, and slope, while showing a positive correlation with herb density and grass density on axis 2. Additionally, in the Kardhai-mixed habitat, species including *Anogeissus pendula*, *Cassia fistula*, *Terminalia arjuna*, *Gardenia latifolia*, and *Acacia latifolia* demonstrated a positive relationship with altitude, distance near human habitation, and slope.

Density and status of regeneration: The study carried out within a 2.653-hectare area on density of trees and the regeneration density of seedlings and saplings (Table 1). The

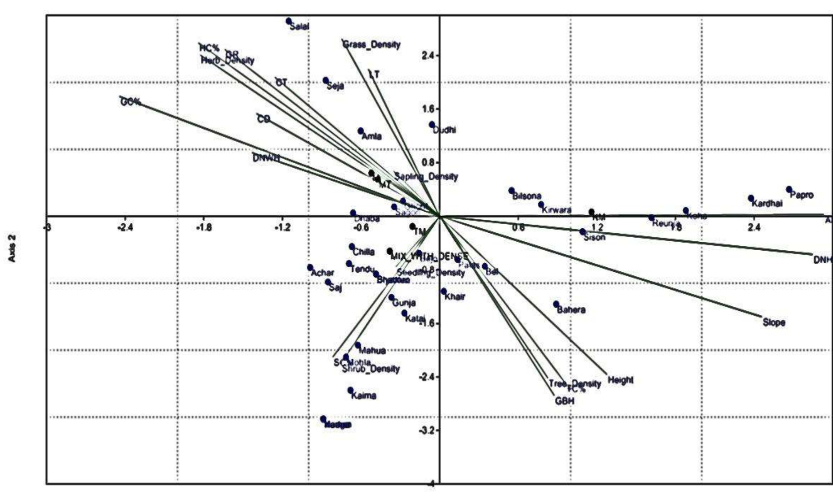

Fig. 2. Canonical correspondence analysis (CCA) ordination diagram, showing the influence of predictive factors on tree- species assemblages in each habitat (A). winter (B). summer. Arrow symbolize the predictive factors. Habitat codes represent the following: *TM* Teak-mixed; *MT* Mixed-thorn; *M* Mixed; *KM* Kardhai-mixed; *Mix with Dense* Mixed with dense understory

Table 1. Regeneration density and status of tree species in Panna tiger reserve

Species	Tree (Individual ha^{-1})	Seedling (Individual ha^{-1})	Sapling (Individual ha^{-1})	Regeneration status
Aegle marmelos	14.227	7.349	13.662	Good
Anogeissus latifolia	15.546	0.659	0.094	Good
Abrus precatorius	13.944	0	0	Non-regenerating
Adina cordifolia	0.376	0	0	Non-regenerating
Anogeissus pendula	11.306	4.711	3.580	Good
Acacia catechu	22.990	1.507	1.224	Good
Acacia latifolia	1.130	0.188	0.188	Good
Allangium salvifolium	0.753	0.094	0	Fair
Albizzia procera	0	0.848	0.188	Good
Azadirachta indica	0.188	0.282	0.282	Good
Balanites aegyptiaca	4.334	1.978	1.413	Good
Bassia latifolia	1.884	0.188	0	Fair
Bauhinia racemosa	2.355	0.753	0.848	Good
Butea monosperma	5.182	0.188	0.848	Good
Boswellia serrata	1.790	0	0	Non-regenerating
Bombax ceiba	0.565	0.094	0	Fair
Buchanania lanzan	0.848	0.188	0	Fair
Ceriscoides turgida	0.376	1.978	0.565	Good
Cassia fistula	2.167	0.659	1.884	Good
Careya arborea	0.094	0	0	Non-regenerating
Diospyros melanoxylon	13.944	16.206	10.647	Good
Euginea jambolana	0.942	3.109	2.826	Good
Feronia elephantum	0.376	0	0.942	Poor
Ficus infectoria	0.471	0	0	Non-regenerating
Ficus benghalensis	0.094	0	0	Non-regenerating
Ficus religiosa	0.188	0	0	Non-regenerating
Gmelina arborea	0.282	0	0.094	Poor
Holoptelea integrifolia	0.094	0	0.094	Poor
Limonia acidissima	1.507	3.015	3.768	Good
Lannea coromandelica	0.188	0	0	Non-regenerating
Lagerstroemia parviflora	18.656	2.544	6.124	Good
Moringa oleifera	0.094	0	0	Non-regenerating
Mangifera indica	0.188	0	0	Non-regenerating
Phyllanthus emblica	2.449	0.094	0	Fair
Pterocarpus marsupium	0.094	0	0	Non-regenerating
Stephegyne parvifolia	2.167	0	0.471	Poor
Saccopetalum tomentosum	1.413	0	0	Non-regenerating
Sterculia urens	0.471	0	0	Non-regenerating
Schleichera trijuga	0.188	0	0	Non-regenerating
Soymida febrifuga	0.376	0	0	Non-regenerating
Tamarindus indica	0.094	0	0	Non-regenerating
Terminalia arjuna	0.942	0.094	0	Fair
Tectona grandis	56.533	5.276	19.032	Good
Terminalia tomentosa	1.696	1.319	0	Fair
Terminalia bellirica	0.565	0	0	Non-regenerating
Wrightia tintoria	1.507	6.784	11.118	Good
Zizyphus xylopyra	19.692	10.081	8.951	Good
Total	225.280	70.288	89.038	

Tectona grandis had the highest species density, with 56.53 individuals per hectare, indicating a rich presence of this species in the area followed by *Acacia catechuZizyphus xylopyra*, *Lagerstroemia parviflora*, *Anogeissus latifolia*, and *Aegle marmelosa. Moringa oleifera*, *Ficus benghalensis*, *Pterocarpus marsupium*, *Holoptelea integrifolia*, and *Careya arborea* exhibited the lowest species density, each with only 0.094 individuals per hectare.The seedling density was significantly lower than in previous studies carried out in the tropical dry deciduous forest (Pawar et al., 2012, Bargali et al., 2014, Kothadaraman and Sundarapandian 2017, Phongoudome et al., 2012). The density of saplings was also lower when compared with findings from previous research (Bargali et al.,2014 , Phongoudome et al., 2012). This discrepancy underscores a significant reduction in forest regeneration. The number of young trees within a certain area directly shows how the local environment affects forest density.

Nineteen species demonstrated robust regenerative capabilities, successfully establishing themselves as trees, seedlings, and saplings. These species collectively represented 41.304 percent of all tree species. The *Diospyros melanoxylon* and *Tectona grandis*, exhibited superior regenerative capacities within this locale. The significant portion of tree species (15.217 percent) observed in tree and seedling stages, were categorized as having fair status. Additionally, 36.956 percent of tree species were identified as challenging to establish within the community, as they were exclusively represented in adult or tree stages, rendering them non-regenerative.The four tree species – *Holoptelea integrifolia*, *Stephegyne parviflora*, *Feronia elephantum*, and *Gmelina arborea* were identified as poor regenerators. They were only found in tree and sapling forms, not as seedlings. These findings could help policymakers make well-informed decisions regarding selecting and managing tree species within the

Table 2. CCA axis lengths of tree-species showing the level of correlation between CCA scores and environmental factors. (Eigen value and % variance of species-environment relationship)

	Tree			
	Axis 1	Axis 2	Axis 3	Axis 4
Canonical eigen value	0.44^{ns}	0.17^{ns}	0.091	0.048
Cumulative % variance	58.36%	22.85%	12.3%	6.50%
Correlation coefficient				
Slope	0.820	-0.500	0.413	-0.100
Height	0.425	-0.788	0.108	-0.707
GBH	0.291	-0.892	-0.009	-0.598
Tree density	0.275	-0.806	-0.083	-0.704
Seedling density	-0.111	-0.252	0.059	-0.960
Sapling density	-0.117	-0.224	-0.710	-0.442
Shrub density	-0.250	-0.732	0.534	-0.137
Herb density	-0.609	0.803	-0.326	0.372
Grass density	-0.248	0.882	-0.428	0.364
Shrub cover%	-0.271	-0.701	0.568	-0.191
Tree cover%	0.320	-0.832	-0.149	-0.639
DNWH	-0.477	0.317	0.256	0.823
Altitude	0.978	0.007	-0.067	-0.127
CT	-0.419	0.693	-0.128	0.801
LT	-0.182	0.732	0.477	-0.419
CD	-0.466	0.510	0.201	0.810
GR	-0.547	0.830	-0.124	0.562
Herb cover%	-0.613	0.862	-0.159	0.313
Grass cover%	-0.814	0.596	-0.345	0.214
DNHH	0.949	-0.189	-0.253	0.104

DNWH= Distance near to waterbody; DNHH= Distance near to human habitation; CT= cutting tree; LT= Lopping tree; CD= Cattle dung; GR= Grazing; ns= Not significant community.After conducting comprehensive CCA, occurrence of specific non-regenerating tree species, specifically *Scheichera trijuga*, *Careya arborea*, *Adina cordifolia*, *Holoptelea integrifolia*, *Abrus precatorius*, *Stephegyne parvifolia*, and *Terminalia bellirica*, were negatively correlated with both the presence of shrub cover and tree cover.

The forest encompasses a diverse range of commercially valuable tree species. Indigenous communities residing within or in close proximity to these forested areas depend on these tree species for their sustenance. The leaves of *Diospyros melanoxylon*, fruits of *Eugenia jambolana*, *Ficus* spp, *Terminalia bellirica*, and *Bassia latifolia* flowers are harvested during May and June. However, potential repercussions of the prolonged harvesting of these invaluable tree speciesmay impede their regrowth and contribute to a decline in forest cover and biodiversity.

Principal Component Analysis

Trees: The PCA was used to examine 17 environmental parameters across 338 plots with the aim of identifying critical environmental factors that support varying tree species density.

The dataset of this study successfully passed the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity, indicating the suitability of conducting PCA ($F_{2,136}$ = 1014.81, P<0.001). The PCA ordination results indicate that three principal components with an eigenvalue loading greater than 1 were extracted, explaining 36.14 percent of the total variance in the original dataset (Table 3). The first principal component (PC1), associated with habitats exhibiting higher frequencies of lopping trees, chopping trees, and increased cattle grazing, displayed a positive correlation with disturbance variables, explaining 14.81 percent of the variance. The second principal component (PC 2) demonstrated a positive relationship with herb and grass density, whereas the third principal component (PC 3) showed a positive association with shrub density and shrub cover, suggesting that there was a significant vertical stratification impact.

Seedling and sapling: The loading plot of the rotated

Table 3. Principal component analysis of environmental variables for tree, seedling and sapling density

	Tree density			Seedling density			Sapling density		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
Eigen Value	2.52	1.86	1.78	3.01	2.09	1.84	2.93	2.08	1.87
Variance (%)	14.81	10.91	10.42	17.67	12.24	10.79	17.61	12.18	10.95
Cumulative variance (%)	14.81	25.71	36.13	17.67	29.89	40.69	17.61	29.78	40.72
Variables									
Shrub Density	-4.26×10^{-2}	-4.10×10^{-2}	**0.918**	-0.326	**0.790**	0.120	-0.339	0.133	**0.694**
Herb Density	5.04×10^{-3}	**0.891**	1.641×10^{-2}	-0.157	-0.100	**0.765**	-0.249	**0.682**	0.185
Grass Density	-2.25×10^{-2}	**0.886**	7.121×10^{-2}	-0.290	-0.242	**0.773**	-0.109	**0.787**	1.98×10^{-2}
Herb Cover (%)	0.122	-8.37×10^{-2}	-5.14×10^{-3}	0.279	0.369	-0.108	0.140	-0.238	0.234
Grass Cover (%)	-5.37×10^{-2}	1.52×10^{-2}	4.970×10^{-2}	-0.139	-0.151	8.929× 10-2	-0.210	0.262	-0.431
Shrub Cover (%)	-0.118	0.127	**0.909**	-0.436	**0.672**	0.367	-0.430	0.241	**0.710**
Tree Cover (%)	-3.84×10^{-2}	-0.287	0.161	7.342×10^{-2}	0.427	-0.475	2.827×10^{-2}	-0.630	0.281
Altitude	-3.16×10^{-2}	0.104	8.792×10^{-2}	8.074×10^{-2}	0.124	**0.524**	0.147	0.445	4.366×10^{-2}
Slope (%)	8.557×10^{-2}	-1.58×10^{-2}	-2.75×10^{-2}	-4.07×10^{-2}	-0.325	8.144 ×10-2	-2.45×10^{-2}	9.835×10^{-2}	-0.337
DNWH	0.247	1.031×10^{-2}	4.462×10^{-2}	0.369	0.314	0.207	0.492	-8.03×10^{-2}	0.427
DNHH	-0.109	9.872×10^{-2}	7.574×10^{-2}	-0.461	-8.74 ×10-2	8.849× 10-2	-0.341	9.256×10^{-2}	-0.349
Aspect	-0.170	0.212	8.661×10^{-2}	-0.234	0.185	7.512 ×10-2	-0.220	-0.185	7.434×10^{-2}
Cutting Tree	**0.648**	2.064×10^{-3}	-2.10×10^{-2}	**0.719**	0.197	0.179	**0.745**	0.157	0.164
Lopping Tree	**0.801**	4.496×10^{-2}	4.768×10^{-2}	**0.787**	0.111	0.293	**0.763**	0.178	0.202
Cattle Dung	**0.721**	-4.21×10^{-2}	-0.190	**0.659**	-0.168	9.636 ×10-2	**0.722**	0.117	-0.155
Grazing	**0.703**	-3.88×10^{-2}	-5.62×10^{-2}	**0.695**	8.433× 10-2	0.198	**0.669**	0.200	-5.792×10^{-2}
Fire	-1.78×10^{-2}	-0.139	-9.66×10^{-2}	0.167	-2.32 ×10-2	2.225 ×10-2	0.139	$5.499\ 10^{-2}$	3.090×10^{-2}

DNWH= Distance Near Waterbody, DNHH= Distance Near Human Habitation.
Bold letter indicates higher correlation between variables and its corresponding principal component

principal components revealed the recovery of three components with an eigenvalue loading of one, collectively explaining 40.7 percent of the total variance in the seedling dataset (Table 3). The second principal component (PC 2) in the multivariate analysis showed a substantial loading of 2.09 eigen values, explaining approximately 12.24% of the total variance. This component demonstrated a strong association with shrub density and shrub cover, which were positively correlated. Furthermore, the third principal component (PC 3) explained 10.79% of the total variance, with two environmental factors showing significant positive associations with this component.In the sapling data set, four environmental variables exhibited a strong positive correlation and significantly contributed to the loading of the first principal component (PC1). These variables, indicative of disturbance, collectively represented an eigen value loading of 3.003 and accounted for 17.67 percent of the total variance (Table 3). In the primary component (PC 1), all disturbance variables in the sapling data set demonstrated a positive correlation, responsible for 17.61% of the total variance. Furthermore, two environmental variables exhibited a positive correlation with the second primary component (PC 2), while another variable showed a negative correlation. Herb density, grass density, and tree cover (%) contributed 12.18% to the overall variance. The third principal component (PC 3) positively and significantly correlated with biotic factors such as shrub density and shrub cover.

Multiple Linear Regression

Trees: The study utilized Multiple Linear Regression to identify the optimal linear combination of Principal Component (PC) scores for the prediction of tree density.

In the analysis, Model 1 demonstrated an ability to account for 55% of the variability in tree density, as indicated by the corrected R^2 value of 0.55. Specifically, in Model 1, where the principal component score was utilized as the independent variable, it was observed that only PC 3 was selected as a significant factor in explaining the variability in tree density (R = 0.397). This finding suggests that tree density could be effectively predicted using only PC 3 as a predictor in Model 1.

Tree density 1 = 15.700 – 0.626 (PC 3)

Considering the correlation coefficient of PC 3 in model 1 and the associated PCA outcomes, which indicate a strong correlation between PC 3 and two specific variables, it is reasonable to infer that a reduction in both shrub density and shrub cover will correspond to an increase in tree density.The adjusted R^2 score of 0.80 for Model 2 indicates its ability to account for 80% of the variation in tree density. In contrast to Model 1, both PC 3 and PC 1 significantly contribute to the explanation of tree density ($P<0.01$, R = 0.430). Moreover, in Model 2, PC 1 emerges as a crucial factor in replicating tree density. The final Model 2 is described as follows (Table 4):

Tree density 2 = 15.700 - 0.626 (PC 3) – 0.401 (PC 1)

The model provided accurate predictions regarding tree density while elucidating the environmental factors influencing it. Shrub density, shrub cover, and various forms of disturbance emerged as the primary ecological variables impacting tree density within the studied area. Notably, these variables exhibited a clear inverse relationship.

Table 4. Results of regression analysis for tree, seedling and sapling density

Independent variables	Regression coefficient		Standard coefficient	t	P	Tolerance	VIF	Adjusted R^2
Tree density								
	B	Std. error	B					
Constant	15.71	0.293		53.54	0.000			
PC 3	-0.626	0.294	0.416	8.11	0.000	1.000	1.000	0.55
Constant	15.71	0.292		53.54	0.000			
PC 3	-0.646	0.292	0.420	8.22	0.000	0.999	1.001	0.80
PC 1	-0.401	0.292	-0.109	-2.15	0.033	0.999	1.001	
Seedling density								
Constant	20.15	0.802		28.88	0.000			
PC 2	-2.67	0.804	-0.250	-3.33	0.001	1.000	1.000	0.57
Sapling density								
Constant	23.54	0.779		30.19	0.000			
PC 2	-2.55	0.782	-0.236	-3.26	0.001	1.000	1.000	0.44

Aegle marmelos and *Wrightia tinctoria*, two of the top five seedling and sapling species, failed to establish themselves as dominant adult tree species. *Wrightia tinctoria* exhibited higher prevalence in disturbed areas receiving direct sunlight, whereas *Aegle marmelos* displayed associations with areas characterized by dense canopies. The species *Wrightia tinctoria*, *Phyllanthus emblica*, *Ceriscoides turgida*, *Acacia catechu*, and *Lagerstroemia parviflora* were demonstrated an association with disturbance gradients, such as the presence of cattle dung and lopped trees. These disturbances lead to an increased exposure to direct sunlight due to the openness of the Mixed habitat type.The forest peripheries, where *Lagerstroemia parviflora* and *Butea monosperma* were prevalent, exhibited distinct environmental conditions. These areas experienced higher air temperatures, reduced humidity, and lower soil moisture levels compared to the forest interior. These conditions create an environment conducive to the growth of species that depend on ample light for their development (Chazdon et al., 1998, Marod et al., 2010, Fayiah et al., 2018). *Lagerstroemia parviflora* possesses a remarkable capability to produce a wide range of seeds within a single individual, contributing to its successful germination across diverse environmental conditions (Shukla and Ramakrishnan 1981). This inherent adaptability has rendered *Lagerstroemia parviflora* well-suited to thrive in the unique conditions of forest edges, making it an intriguing subject for further research and study.In contrast, *Diospyros melanoxylon* was identified as the tree species with the highest germination rate, and it can tolerate higher shrub cover percentages. Khurana and Singh (2000) have observed a unique biological feature of *Diospyros melanoxylon*. This feature allows the species to delay the germination process until the following rainy season if there is insufficient soil moisture during initial germination. This adaptation enables the species to survive and thrive in fluctuating environmental conditions.

Seedling: The principal component scores significantly affected seedling density. The significant value of PC 2 was less than 0.05, indicating significance and influence on seedling density. Moreover, the modified R^2 value of 0.57 suggests that the independent variables explain 57% of the dependent variable, seedling density. The multiple regression equation is as follows (Table 4):

Seedling density = 20.15 – 2.669 (PC 2)

Principal Component 2 (PC 2) showed a statistically significant relationship with both shrub density and shrub cover. this component exhibited a negative correlation with these variables. The highest shrub density was recorded for *Lantana camara*, which is categorized as alien weeds (Fig. 3). In addition, the analysis of land use and land cover revealed that the predominant habitat type within the Tiger reserve area was classified as mixed with dense understory. The understory of this habitat type was explicitly characterized by the dense presence of *Lantana camara*. The adaptability of *Lantana* was also observed across various habitat types, including Teak-mixed, Mixed, and Kardhai-mixed, except the Mixed-thorn habitat. This versatile invader may potentially impede the natural regeneration of native trees, ultimately leading to a decline in both tree diversity and the overall structural integrity of the ecosystem. The *Lantana* invasion has been the subject of extensive research due to its significant impact on the vegetation structure of ecosystems. Previous studies have highlighted that the presence of *Lantana* can induce alterations in soil properties and the hydrology of the affected area. This specific study conducted an assessment and determined that the density of *Lantana* in the area was estimated at 754 individuals per hectare, aligning closely with the broader range of 1500-3000 individuals per hectare typically observed in tropical and sub-tropical forested regions (Joshi 2002).

Sapling: The resultant adjusted R^2 value was calculated to be 0.44, signifying that the model can elucidate 44% of the variance in sapling density. Within the regression model, only PC 2 was identified as a significant explanatory variable for the fluctuations in sapling density ($P<0.01$, $R = 0.236$). The ensuing multiple regression equation is as follows (Table 4):

Sapling density = 23.536 – 2.542 (PC 2)

PC 2 is strongly associated with three specific factors: herb density, grass density, and Tree Cover (%). Even though these variables were have negative relationships, an increase might lead to a decrease in sapling density. The comparative analysis was conducted in 338 plots within the Panna Tiger Reserve to assess the mean density (ha) of the alien weed *Hyptis suaveolens* in relation to native herb species (Fig. 4). This invasive species poses a threat to the local vegetation community (Sharma et al., 2007). The estimated density of *H. suaveolens* was 14906 individuals per hectare. The highest herb density was observed in the Mixed and Mixed-thorn habitat types. Moreover, the species was found near the Gangau B and Naranan beats.The maximum height of *H. suaveolens* typically ranges from 0.5 to 1.5 meters. However, during the growing season reach up to 2.5 meters.Their investigation revealed that *H. suaveolens* exhibited maximum presence in grass-dominated areas, potentially leading to a reduction in grass abundance and subsequently diminishing the available food resources for animal species, particularly small-sized antelopes. Additionally, the study observed that *Hyptis suaveolens* also provides cover for the Four-horned Antelope. These

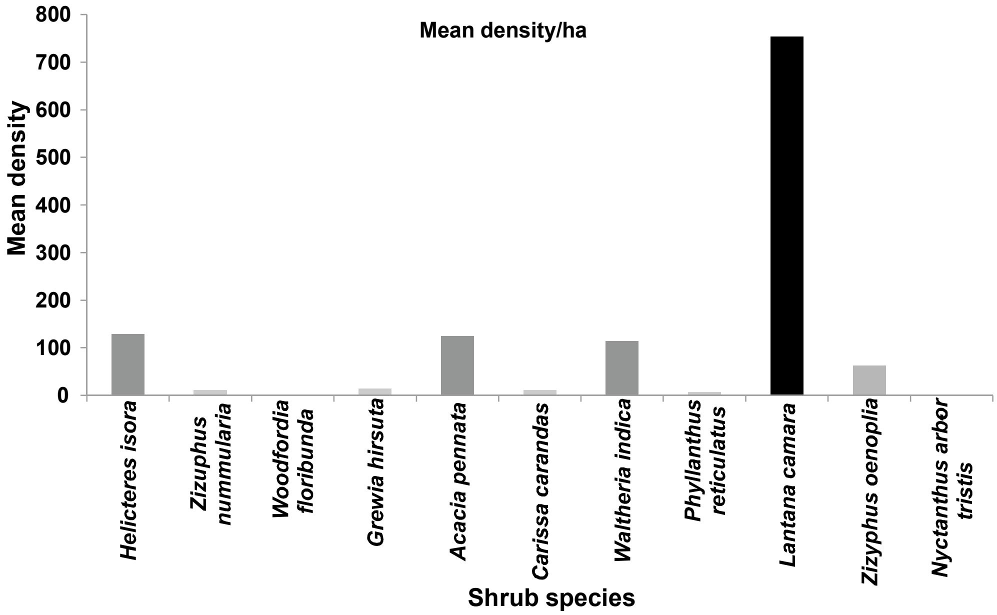

Fig. 3. Mean density (ha) of *Lantana camara*, the alien weed, compared to native shrub species in Panna Tiger Reserve

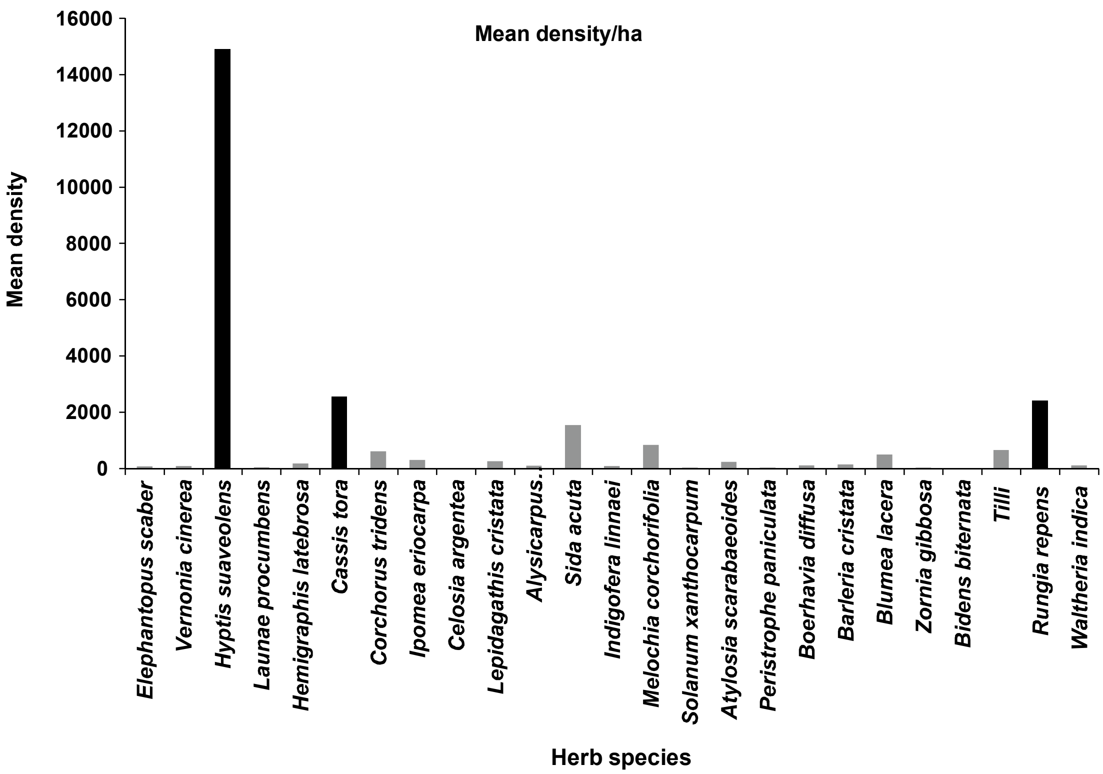

Fig. 4. Mean density (ha) of *Hyptis suaveolens*, the alien weed, compared to native herb species in Panna Tiger Reserve

variables exert significant influence on the process of seed germination and subsequent survival for these particular species. Therefore, it is paramount to incorporate these species into the examination of local ecology. It is important to note that these factors possess the potential to precipitate the local extinction of certain species. Consequently, in-depth investigations of the local ecology should comprehensively address these factors to ensure the preservation of endangered species.

CONCLUSION

The study indicates that the woodland area is at risk of deteriorating into degraded savanna grassland within the coming year. To protect tree species and maintain the region's biodiversity, it is vital to take constructive steps to address these challenges. The research findings also highlight the significant role of *Tectona grandis* in maintaining the ecological balance of the Teak-mixed habitat. In the study area, the unique conditions under the teak canopy supported the successful germination and growth of seedlings of *Aegle marmelos*, *Bassis latifolia*, *Bauhinia racemosa*, *Gardenia latifolia*, *Tectona grandis*, and *Albizzia procerain*. The preservation of this species is crucial not only for its own survival but also for the overall well-being and survival of other species that depend on this specific habitat.The results of this study are significant, as they can be used to bolster the relative importance of Panna Tiger Reserve in regional and local conservation planning. Specifically, these findings can help inform the development of targeted restoration techniques and identify forest communities that require protection.

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Assessment of Pentatomoidea in Dalma Wildlife Sanctuary, Jharkhand, India: Faunal Composition, Abundance and Distribution

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Abstract: This study documents new distributional record of Pentatomoidea species along with their relative abundance in Dalma Wildlife Sanctuary, Jharkhand. The Pentatomoidea, a remarkably diverse group encompassing over 8000 species across 1400 genera globally, is the focus of our research. The 22 species from 18 genera and 3 families (Cydnidae, Pentatomidae, and Scutelleridae) of Pentatomoidea in Dalma Wildlife Sanctuary were recorded which were undocumented. Among these, 16 species representing 13 genera and 3 families constitute the first record for state of Jharkhand.

Keywords: Hemiptera, New record, Pentatomoidea, Relative abundance, Wildlife sanctuary

Pentatomoidea is a superfamily belonging to the insect order Hemiptera, suborder Heteroptera, and the infraorder Pentatomomorpha. They greatly vary in size with the smallest species not much larger than a pin head (*Megaris* Stål in family Megarididae and *Sepontia* Stål in the family Pentatomidae) to the largest and most robust species in the Heteroptera (some members of family Tessaratomidae) (Rider 2018). Most of the species belong to this superfamily are phytophagous, causing economic damage to various crops and a few groups are suspected to be fungivores (Canopidae, Megarididae, some Plataspidae) (Rider 2018). Among the members of this superfamily only subfamily Asopinae under the family Pentatomidae exhibit predatory feeding habits and known as predatory stink bugs. These predatory bugs mainly predate on larvae of lepidopteran, dipteran, and coleopteran insects, both in the juvenile and adult stages (De Clercq 2000).

The superfamily Pentatomoidea comprises with 18 families worldwide of which only 9 families viz., Acanthosomatidae, Cydnidae, Dinidoridae, Parastrachiidae, Pentatomidae, Plataspidae, Scutelleridae, Tessaratomidae, Urostylididae were reported from India (Distant 1902, Lis 2006, Rider 2006, Scheiding 2006, Vilimova 2006). Over 768 species of the superfamily Pentatomoidea have been reported in India, out of about 8042 species known worldwide (Biswas et al., 2014, Rider 2018, Praveen et al., 2024). During the survey conducted at the Chota Nagpur Plateau in Jharkhand state, 22 species belonging to 18 genera and 3 families (Cydnidae, Pentatomidae, and Scutelleridae) within the superfamily Pentatomoidea were documented from the Dalma Wildlife Sanctuary. This study aims to understand the diversity, abundance, and distribution of species belonging to superfamily Pentatomloidea within the Dalma Wildlife Sanctuary in Jharkhand, India. This information is crucial for assessing the ecological health of the sanctuary, as these insects play vital roles in the ecosystem as both predators and herbivores.

MATERIAL AND METHODS

Dalma wildlife sanctuary is located in the East Singhbhum and Sariakela-Kharsawan districts of Jharkhand on the ranges of Dalma Hills and spread out over an area of 193.22 Km^2 covering 86 villages. Dalma Hills are located at a height of 3000 ft above sea level with dense forests and Subarnarekha River (Ranjan et al., 2016). The forests Dalma are classified as northern dry mixed deciduous forest and dry peninsular sal. The majority of woods in this forest loses their leaves in the summer and reach their peak bloom at the start of the monsoon season.

Specimens were collected during 2020-2021 from four sampling sites of Dalma Wildlife Sanctuary (Fig. 5) (spatially located in the East Singhbhum and Sariakela-Kharsawan districts of Jharkhand and spread out over an area of 193.22 Km^2 covering 86 villages), labeled as Site A (Makulakocha Guest House, 22.547˚N, 86.082˚E), Site B (Rugri village, 22.856˚N, 86.1621˚E), Site C (Makulakocha village, 22.9119˚N, 86.1491˚ E) and Site D (Pindrabera Guest House, 22.89363˚N, 86.19637˚E) using the sweep net and light trap methods (Fig. 1).

The sampling site of Pindrabera is situated at higher elevation (200 meters) compared to those at Makulakocha and Rugri (129 meters). During the sampling period, the vegetation at Pindrabera was predominantly composed of trees such as Indian butter tree (*Madhuca longifolia* (L.) J. F. Macbr.), lac tree

(*Schleichera oleosa* (Lour.) Oken), Neem (*Azadirachta indica* Adr. Juss.), yellow teak tree (*Adina cordifolia* (Roxb.) Brandis), Golden Rumph's fig (*Ficus rumphii* Bl.), Indian frankincense tree (*Boswellia serrata* Roxb.), Sal (*Shorea robusta* Gaertn.), Mango (*Mangifera indica* L.), Indian blackberry (*Syzygium cumini* (L.) Skeels). In contrast, the sampling sites at Makulakocha and Rugri exhibited a mixed vegetation cover, characterized by a diverse assemblage of shrubs and herbs (Fig. 2). Collected specimens were brought to the laboratory and then the genitalia of male specimens were dissected (Ahmad (986), for proper identification with the help of suitable literature. The photographs were taken under a Leica M205A stereomicroscope using a Leica DMC-4500 camera. The photographs were processed in LAS V4.12 software. Photographs were edited using Adobe Photoshop CS (Version 8.0).

Relative abundance calculation: The relative abundance (p_i) is the proportional representation of a species in a community or sample of a community. The relative abundance (p_i) of each species is expressed as

$$Pi = \frac{ni}{N} \times 100$$

Where, n_i is the number of individuals of the same species and N is the total number of individuals for all species.

RESULTS AND DISCUSSION

A total of 22 species and 18 genera belonging to 3 families, viz., Cydnidae (1 species), Pentatomidae (19 species) and Scutelleridae (2 species) from superfamily Pemtatomoidea were collected and identified using morpho-taxonomic approach. The taxonomic hierarchy of the identified species from the study area is as follows:

Order Hemiptera
Suborder Heteroptera
Infraorder Pentatomorpha
Superfamily Pentatomoidea
Family Pentatomidae
Subfamily Pentatominae
Tribe: Agonoscelidini

Agonoscelis nubilis (Fabricius 1775) (Fig. 1a)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Rugri village, 22.856˚N, 86.1621˚ E, 10.07.2021, 1♂, Chitra. J and Party.

Distribution in India: Bihar, Jammu & Kahsmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Nagaland, Meghalaya, Orissa, Puducherry, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal (Atkinson 1888, Azim 2011, Chakraborty et al., 1994, Chatterjee 1934, Distant 1902, Fletcher 1920, Salini and Viraktamath 2015), Jharkhand (New record).

Distribution outside India: China, Japan Malayan Peninsula, Myanmar, Pakistan, Sri Lanka (Distant 1902).

Remarks: This species has been recorded for the first time from Jharkhand.

Tribe-Antestiini

Plautia crossota (Dallas 1851) (Fig. 1b)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Rugri village, 22.856˚N, 86.1621˚ E, 10.07.2021, 1♂, Chitra. J and Party.

Distribution in India: Andaman and Nicobar Is, Assam, Bihar, Karnataka, Kerala, Maharashtra, Meghalaya, Nagaland, Orissa, Punjab, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal. (Atkinson 1887, Azim 2011, Chakraborty et al., 1994, Chatterjee 1934, Distant 1902, Ferrari 2009, Hegde 1995, Mathew 1986, Salini 2006), Jharkhand (new record).

Distribution outside India: Afghanistan, China, Japan, Gambia to Sudan, Madagascar, Yemen (Rider 2006).

Remarks: This species has been recorded for the first time from Jharkhand.

Tribe Carpocorini

Aeliomorpha lineaticollis (Westwood 1837) (Fig. 1c)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha village, 22.9119˚N, 86.1491˚ E, 10.07.2021, 1♂, Chitra. J and Party.

Distribution in India: Andhra Pradesh, Bihar, Karnataka, Maharashtra, Tamil Nadu, South India, West Bengal (Atkinson 1887, Chatterjee 1934, Distant 1902, Distant 1918, Salini 2006, Salini 2015), Jharkhand (New record).

Distribution outside India: Afghanistan, China, Syria, tropical Africa (Rider 2006).

Remarks: This species has been recorded for the first time from Jharkhand.

Tribe- Eysarcorini

Carbula insocia Walker 1868 (Fig. 1d)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha village, 22.9119˚N, 86.1491˚ E, 10.07.2021, 1♂, Chitra. J and Party.

Distribution in India: Chhattisgarh, Himachal Pradesh, Karnataka, Kerala, Maharashtra, Tamil Nadu, North West Himalaya (Distant 1902, Hassan et al., 2019, Hegde 1986, Kaur et al., 2013, Mathew 1986, Usman and Puttarudriah 1955), Jharkhand (New record).

Distribution outside India: Pakistan (Kaur et al., 2012).

Remarks: This species has been recorded for the first time from Jharkhand.

Tribe -Menidini

Menida formosa (Westwood 1837) (Fig. 1e)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha village, 22.9119˚N, 86.1491˚ E, 10.07.2021, 1 ♀, Chitra. J and Party.

Distribution in India: Assam, Karnataka, Kerala, Maharashtra, Sikkim, Tamil Nadu, Uttarakhand, West Bengal (Atkinson 1887, Chatterjee 1934, Distant 1902, Salini 2006, Salini and Viraktamath 2015, Usman and Puttarudriah,1955), Jharkhand (New record).

Distribution outside India: China, Malayan Peninsula, Myanmar, Taiwan (Distant 1902, Rider 2006).

Remarks: This species has been recorded for the first time from Jharkhand.

Eysarcoris aenescens (Walker 1867) (Fig. 1f)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Rugri village, 22.856˚N, 86.1621˚ E, 10.07.2021, 1 ♂, Chitra. J and Party.

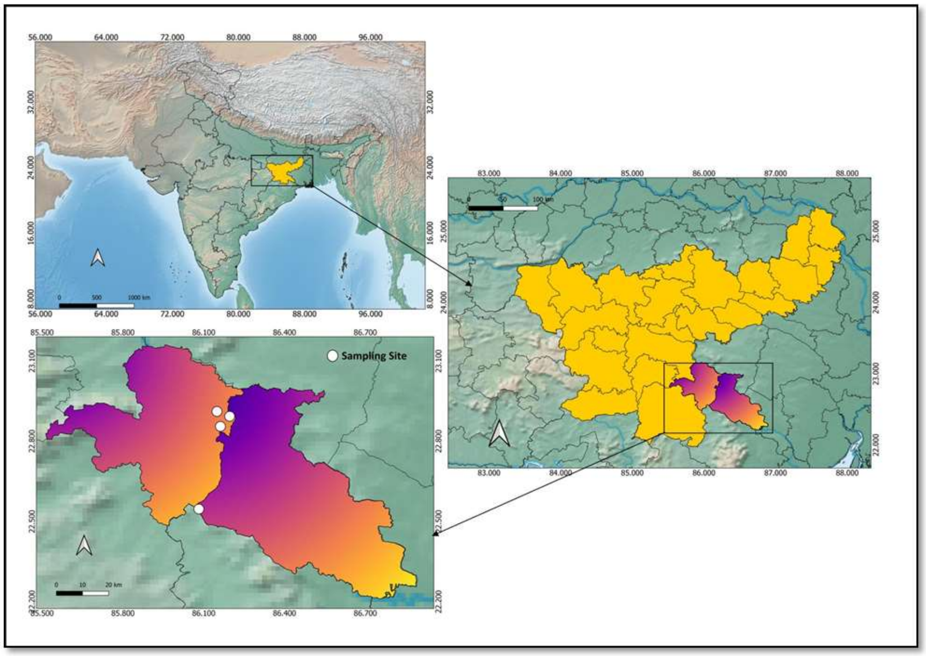

Fig. 1. Sampling sites within Dalma WLS belonging to Saraikela Kharsawan and East Singbhum district of Jharkhand (Bottom)

Fig. 2. Vegetation type at different sampling sites

Distribution in India: Karnataka, Kerala (Salini 2015)

Distribution outside India: China, Indonesia (Rider 2006).

Remarks: This species has been recorded for the first time from Jharkhand.

Eysarcoris guttiger (Thunberg 1783) (Fig. 1g)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Rugri village, 22.856˚N, 86.1621˚ E, 10.07.2021, 1♂, Chitra. J and Party.

Distribution in India: Assam, Bihar, Gujarat, Karnataka, Maharashta, Nagaland, Orissa, Punjab, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal (Atkinson 1887, Azim and Shafee 1984, Chakraborty et al., 1994, Chatterjee 1934, Distant 1902, Distant 1918, Fletcher 1920, Kaur et al., 2012, Salini 2006), Jharkhand (New record).

Distribution outside India: China, Japan Korea, Myanmar, Sri Lanka; Taiwan (Distant 1902, Rider 2006).

Remarks: This species has been recorded for the first time from Jharkhand.

Eysarcoris montivagus Distant 1902 (Fig. 1h)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Rugri village, 22.856˚N, 86.1621˚ E, 10.07.2021, 1♂, Chitra. J and Party.

Distribution in India: Andhra Pradesh, Assam, Gujarat, Kerala, Meghalaya, Nagaland, Punjab, Tamil Nadu, Uttar Pradesh, West Bengal (Distant 1902, Kaur et al., 2012, Mathew 1986, Salini 2006, Salini 2015), Jharkhand (New record).

Distribution outside India: China, Japan Korea, Myanmar, Sri Lanka; Taiwan (Distant 1902, Rider 2006).

Remarks: This species has been recorded for the first time from Jharkhand.

Eysarcoris rosaceus Distant 1901 (Fig. 1i)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Rugri village, 22.856˚N, 86.1621˚ E, 10.07.2021, 2♂, Chitra. J and Party.

Distribution in India: Himachal Pradesh, Punjab, Uttarakhand (Kaur et al., 2012), Jharkhand (New record).

Distribution outside India: China, Myanmar; Pakistan (Rider 2006, Kaur et al., 2012)

Remarks: This species has been recorded for the first time from Jharkhand.

Eysarcoris ventralis (Walker 1837) (Fig. 1j)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Pindrabera Guest House, 22.89363˚N, 86.19637˚ E, 12.07.2021, 1♂, Chitra. J & Party; India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha village, 22.9119˚N, 86.1491˚ E, 10.vii.2021, 3♀, Chitra. J and Party.

Distribution in India: Arunachal Pradesh, Bihar, Jharkhand, Karnataka, Kerala, Maharashtra, Meghalaya, Odisha, Punjab, Tamil Nadu, Uttar Pradesh, West Bengal (Chakraborty et al., 1994, Distant 1902, Fletcher 1920, Salini 2015).

Distribution outside India: Malayan Peninsula, Myanmar, Whole Palaearctic region, *Ethiopian* (Tropical Africa, Cape Verde Islands), *Nearctic* (Hawaii) (Distant 1902, Rider 2006).

Tribe - Halyini

Halys serrigera Westwood 1837 (Fig. 1k)

Material examined: India: Jharkhand, West Saraikela Kharsawan Dalma WLS, Pindrabera Guest House, 22.89363˚N, 86.19637˚ E, 12.07.2021, 1♀, Chitra. J and Party.

Distribution in India: Jharkhand, Kerala, Maharashtra, Meghalaya, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal (Chakraborty et al., 1994, Keshari and Mahto 2017).

Distribution outside India: Afghanistan, China, Japan, Myanmar, Sri Lanka (Rider 2006).

Erthesina fullo (Thunberg 1783) (Fig. 1l)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha Guest House, 22.547˚N, 86.082˚ E, 08.07.2021, 1♂, Chitra. J and Party.

Distribution in India: Andaman Islands, Andhra Pradesh, Assam, Kerala, West Bengal (Atkinson 1887, Chakraborty et al., 1994), Jharkhand (New record).

Distribution outside India: Afghanistan, China, Japan, Tiwan (Rider 2006).

Remarks: This species has been recorded for the first time from Jharkhand.

Tribe Nezarini

Acrosternum graminea (Fabricius 1787) (Fig. 2a)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha Guest House, 22.547˚N, 86.082˚ E, 08.07.2021, 2♂, Chitra. J and Party.

Distribution in India: Andhra Pradesh, Bihar, Gujarat, Karnataka, Maharashtra, Tamil Nadu, Uttar Pradesh, West Bengal (Atkinson 1887, Azim 2011, Chatterjee 1934, Distant 1918, Hegde 1995, Salini 2006, Salini 2015), Jharkhand (New record).

Distribution outside India: Iraq, Maldive Is., Pakistan, Sri Lanka (Rider 2006).

Remarks: This species has been recorded for the first time from Jharkhand.

Nezara viridula (Linnaeus 1758) (Fig. 2b)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha Guest House, 22.547˚N, 86.082˚ E, 08.07.2021, 2♂, Chitra. J and Party.

Distribution in India: Jharkhand, Maharashtra, Southern Peninsula, West Bengal (Chakraborty et al., 1994, Keshari and Mahto 2017, Chattopadhyay 2021).

Distribution outside India: Asia, Europe, North America (Cosmopolitan) (Rider 2006).

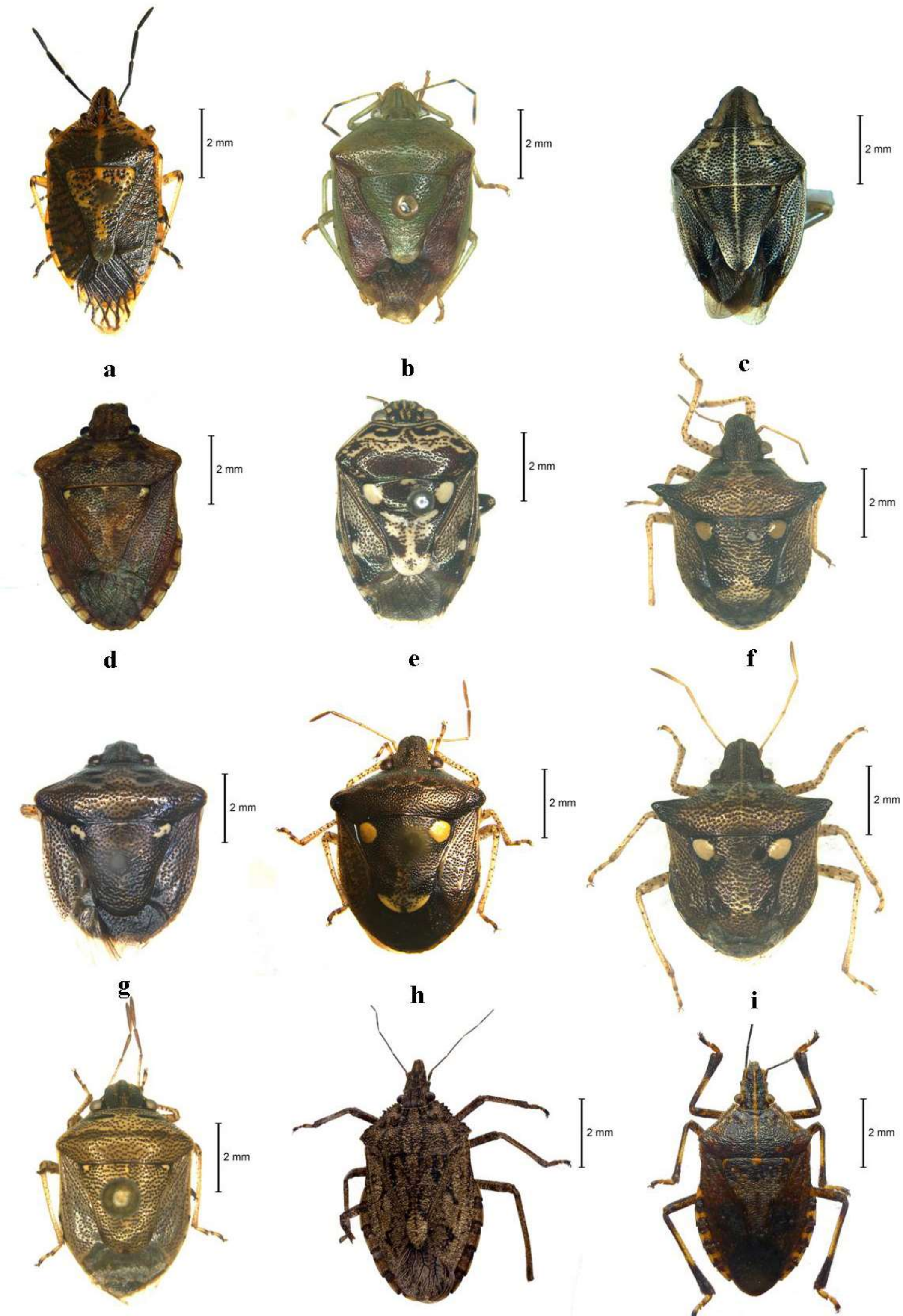

Fig. 3. Habitus. a. *Agonoscelis nubilis* (Fabricius 1775), b. *Plautia crossota* (Dallas 1851), c. *Aeliomorpha lineaticollis* (Westwood 1837), d. *Carbula insocia* Walker 1868, e. *Menida formosa* (Westwood 1837), f. *Eysarcoris aenescens* (Walker 1867), g. *Eysarcoris guttiger* (Thunberg 1783), h. *Eysarcoris montivagus* Distant, 1902, i. *Eysarcoris rosaceus* Distant, 1901, j. *Eysarcoris ventralis* (Walker 1837), k. *Halys serrigera* Westwood 1837, l. *Erthesina fullo* (Thunberg 1783)

Tribe Piezodorini

Piezodorus hybneri Gmelin 1790 (Fig. 2c)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha Guest House, 22.547˚N, 86.082˚ E, 08.07.2021, 2♂, Chitra. J and Party.

Distribution in India: Assam, Maharashtra, Orissa, West Bengal (Chakraborty et al., 1994), Jharkhand (New record).

Distribution outside India: Africa, China, Indonesia, Japan, Tiwan, Yemen (Rider 2006).

Remarks: This species has been recorded for the first time from Jharkhand.

Tribe Strachiini

Bagrada hilaris Burmeister 1835 (Fig. 2d)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha Guest House, 22.547˚N, 86.082˚ E, 08.07.2021, 1♂, 1♀, Chitra. J and Party.

Distribution in India: Andhra Pradesh, Bihar, Himachal Pradesh, Jharkhand, Karnataka, Maharashtra, Punjab, Rajasthan, Tamil Nadu; Uttar Pradesh; Uttarakhand, West Bengal (Atkinson 1887, Chatterjee 1934, Fletcher 1920, Keshari and Mahto 2017, Salini 2006, Salini 2015, Usman and Puttarudriah 1955, Kaur et al., 2012).

Distribution outside India: Afghanistan, Algeria, Cape Verde Islands, China, Egypt, Italy (Pantellaria), Iraq, Israel, Libya, Macedonia, Malta, Morocco, Saudi Arabia, Syria, tropical Africa, Yemen (Rider 2006).

Subfamily Asopinae

Andrallus spinidens (Fabricius 1787) (Fig. 2e)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha Guest House, 22.547˚N, 86.082˚ E, 08.07.2021, 1♀, Chitra. J and Party.

Distribution in India: Assam, Karnataka, Maharashtra, Odisha, Tamil Nadu, Uttar Pradesh, West Bengal (Atkinson 1888, Azim 2011, Azim and Shafee 1982, Chatterjee 1934, Distant 1902, Salini 2006), Jharkhand (New record).

Distribution outside India: Australia, Azerbaijan, Central and South America, China, Greece, Iran, Italy, Japan, southern United States, Tiwan, tropical Africa, Turkey Asian part, Turkmenistan (Rider 2006; Pal et al., 2023)

Remarks: This species has been recorded for the first time from Jharkhand.

Eocanthecona furcellata (Wolff 1811) (Fig. 2f)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha Guest House, 22.547˚N, 86.082˚ E, 08.07.2021, 1♂, Chitra. J and Party.

Distribution in India: Assam, Bihar, Jammu and Kashmir, Jharkhand, Karnataka, Maharashtra, Nagaland, Odisha, Punjab, Tamil Nad, Uttarakhand, West Bengal (Atkinson 1888, Azim 2011, Azim and Bhat 2010, Chatterjee 1934, Datta et al., 1985, Distant 1902, Hegde 1995, Kaur et al., 2012, Salini 2006, Salini and Viraktamath 2015, Usman and Puttarudriah 1955).

Distribution outside India: Bangladesh, China, Japan, Myanmar, Sri Lanka, Taiwan and Thailand (Pal et al., 2023).

Zicrona caerulea (Linnaeus 1758) (Fig. 2g)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha Guest House, 22.547˚N, 86.082˚ E, 08.07.2021, 1♀, Chitra. J and Party.

Distribution in India: Jammu & Kashmir, Nagaland and West Bengal (Chakraborty et al., 1994, Pal et al., 2023), Jharkhand (New record).

Distribution outside India: Afghanistan, Algeria, Austria, Azerbaijan, Belgium, Burma, China, Czechoslovakia, Egypt, England, Finland, France, Germany, Greece, Ireland, Israel, Italy, Japan, Java, Korea, Malaysia, Mongolia, Morroco, Netherlands, Pakistan, Portugal, Russia, Spain, Sumatra, Sweden, Switzerland, Syria, Taiwan, Turkey, Vietnam (Thomas 1994, Pal et al., 2023).

Remarks: This species has been recorded for the first time from Jharkhand.

Family Cydnidae

Aethus indicus (Westwood 1837) (Fig. 2h)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha Guest House, 22.547˚N, 86.082˚ E, 08.07.2021, 1♀, Chitra. J and Party.

Distribution in India: Madhya Pradesh, Maharashtra, Odisha, South India, West Bengal (Lis 1993, Chandra et al., 2015), Jharkhand (New record).

Distribution outside India: Burma, Indonesia, Sri Lanka, Thailand (Lis 1993).

Remarks: This species has been recorded for the first time from Jharkhand.

Family Scutelleridae

Chrysocoris stollii (Wolff1801) (Fig. 2i)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha Guest House, 22.547˚N, 86.082˚ E, 08.vii.2021, 2♀, Chitra. J and Party.

Distribution in India: Andaman & Nicobar Islands, Assam, Bihar, Chhattisgarh, Delhi, Jharkhand, Kerala, Maharashtra, Madhya Pradesh, Meghalaya, Nagaland, Odisha, Sikkim, Telangana, Uttarakhand, Uttar Pradesh and West Bengal (Husain and Dubey 2021).

Distribution outside India: Bangladesh, Cambodia, China, Indonesia, Malaysia, Myanmar, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand and Vietnam (Husain and Dubey 2021).

Hotea curculionoides (Herr-Sch1835) (Fig. 2j)

Material examined: India: Jharkhand, Saraikela Kharsawan, Dalma WLS, Makulakocha Guest House,

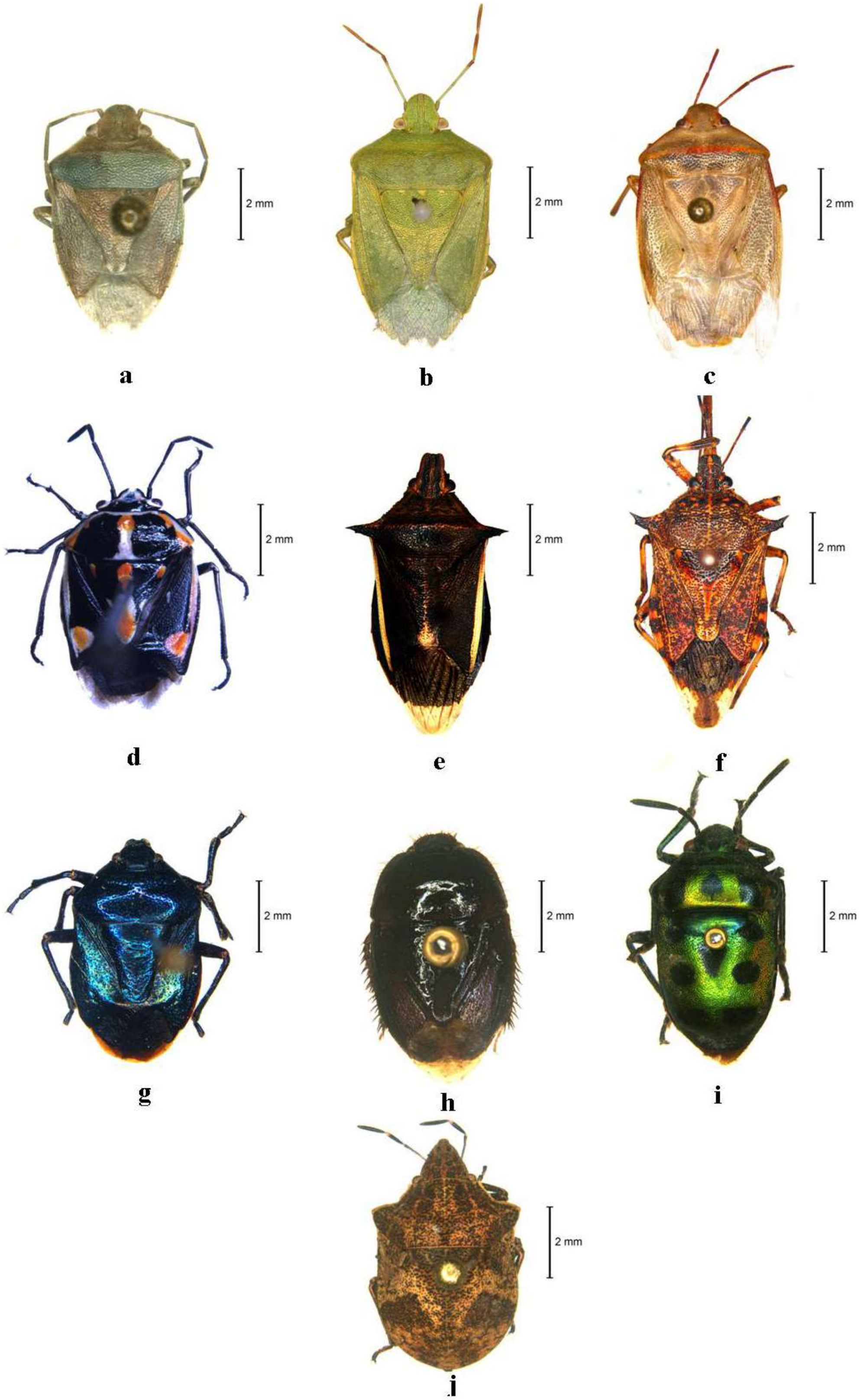

Fig. 4. Habitus. a. *Acrosternum graminea* (Fabricius 1787), b. *Nezara viridula* (Linnaeus 1758), c. *Piezodorus hybneri* Gmelin, 1790, d. *Bagrada hilaris* Burmeister, 1835, e. *Andrallus spinidens* (Fabricius 1787), f. *Eocanthecona furcellata* (Wolff 1811), g. *Zicrona caerulea* (Linnaeus 1758), h. *Aethus indicus* (Westwood 1837), i. *Chrysocoris stollii* (Wolff 1801), j. *Hotea curculionoides* (Herr-Sch1835).

Table 1. Relative abundance of Pentatomoidea fauna from Dalma WLS

Family	Species	Site A		Site B		Site C		Site D		Total	RA
		n_i	RA	n_i	RA	n_i	RA	n_i	RA		
Pentatomidae	*Agonoscelis nubilis* (Fabricius 1775)	0	0	1	14.28	0	0	0	0	1	3.22
	Plautia crossota (Dallas 1851)	0	0	1	14.28	0	0	0	0	1	3.22
	Aeliomorpha lineaticollis (Westwood 1837)	0	0	0	0	1	16.66	0	0	1	3.22
	Carbula insocia Walker 1868	0	0	0	0	1	16.66	0	0	1	3.22
	Menida formosa (Westwood 1837)	0	0	0	0	1	16.66	0	0	1	3.22
	Eysarcoris aenescens (Walker 1867)	0	0	1	14.28	0	0	0	0	1	3.22
	Eysarcoris guttiger (Thunberg 1783)	0	0	1	14.28	0	0	0	0	1	3.22
	Eysarcoris montivagus Distant 1902	0	0	1	14.28	0	0	0	0	1	3.22
	Eysarcoris rosaceus Distant (1901)	0	0	2	28.57	0	0	0	0	2	6.45
	Eysarcoris ventralis (Walker 1837)	0	0	0	0	3	50	1	50	4	12.90
	Halys serrigera Westwood 1837	0	0	0	0	0	0	1	50	1	3.22
	Erthesina fullo (Thunberg 1783)	1	6.25	0	0	0	0	0	0	1	3.22
	Acrosternum graminea (Fabricius 1787)	2	12.5	0	0	0	0	0	0	2	6.45
	Nezara viridula (Linnaeus 1758)	2	12.5	0	0	0	0	0	0	2	6.45
	Piezodorus hybneri Gmelin 1790	2	12.5	0	0	0	0	0	0	2	6.45
	Bagrada hilaris Burmeister 1835	2	12.5	0		0	0	0	0	2	6.45
	Andrallus spinidens (Fabricius 1787)	1	6.25	0	0	0	0	0	0	1	3.22
	Eocanthecona furcellata (Wolff 1811)	1	6.25	0	0	0	0	0	0	1	3.22
	Zicrona caerulea (Linnaeus 1758)	1	6.25	0		0	0	0	0	1	3.22
Cydnidae	*Aethus indicus* (Westwood 1837)	1	6.25	0	0	0	0	0	0	1	3.22
Scutelleridae	*Chrysocoris stollii* (Wolff1801)	2	12.5	0	0	0	0	0	0	2	6.45
	Hotea curculionoides (Herr-Sch1835)	1	6.25	0		0	0	0	0	1	3.22
Total		16	100	07	100	06	100	02	100	31	100

22.547˚N, 86.082˚ E, 08.07.2021, 1♀, Chitra. J and Party.

Distribution in India: Bihar, Delhi, Meghalaya, Nagaland, Uttar Pradesh, West Bengal (Dhali 2021), Jharkhand (New record).

Distribution outside India: China, Hong Kong, Indonesia, Myanmar, Pakistan, Sri Lanka, Vietnam (Dhali 2021).

Remarks: This species has been recorded for the first time from Jharkhand.

This study documents the faunal composition of Pentatomoidea for the first time from Dalma wildlife sanctuary, Jharkhand, India. A total of 31 individuals of superfamily Pentatomoidea were collected from the four sampling sites of Dalma WLS, Jharkhand of which 25.80 % of individuals were collected by using light trap method and 74.19 % of individuals were collected by using sweep net method (Table 1). The 3 families of superfamily Pentatomoidea were represented by 22 species collected. The present study reveals that Pentatomidae is the most abundant family with a relative abundance (RA) of 87.09 %, followed by Scutellaridae and Cydnidae has least abundant family (3.22 %) (Table 1). Numerically the most abundant species is *Eysarcoris ventralis* (Walker, 1837) with a relative abundance of 12.90 %, followed by *Eysarcoris rosaceus,* Distant (1901), *Acrosternum graminea* (Fabricius, 1787), *Nezara viridula* (Linnaeus, 1758), *Piezodorus hybneri* Gmelin, 1790, *Bagrada hilaris* Burmeister, 1835, *Chrysocoris stollii* (Wolff,1801) with a relative abundance (of 6.45%. There are 15 species with the least relative abundance (RA) value of 3.22% (Table 1).

CONCLUSIONS

This study on Pentatomoidea from Dalma Wildlife Sanctuary in Jharkhand, India, has yielded valuable insights into the diversity and distribution of these species in the area. A total of 22 species across 18 genera and 3 families (Cydnidae, Pentatomidae, and Scutelleridae) were identified, with 16 species from 13 genera and 3 families representing the first recorded occurrence in the entire state of Jharkhand. This research underscores the urgent need for conservation efforts to ensure the survival of these species. The findings have created a comprehensive database of Pentatomoidea that will support future initiatives aimed at conserving and enhancing the local biodiversity of these insects.

AUTHORS CONTRIBUTION

S. Dash: Identification of specimens, and writing of the manuscript, A. Pal: Identification of specimens, and writing of the manuscript, P.C. Saha: Identification of specimens

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Identification of Non-Timber Forest Products and Economic Value- A Study in Eastern Himalayan States of India

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Abstract: The study was conducted in the Eastern Himalayan states of Meghalaya and Nagaland, selecting two districts from each state. From twenty villages, 250 respondents were proportionately selected based on household availability. Non-Timber Forest Products (NTFPs) were valuated using the direct use value method and categorized by consumptive uses. In Meghalaya, 17 plant-based and 5 animal-based NTFPs were identified, primarily for nourishment, followed by therapeutic uses, alimentary supplements, fuel wood, housing material, and ornate products. Nagaland identified 16 plant-based and 9 animal-based NTFPs, with similar categories. The average time spent per trip collecting plant-based NTFPs was 2.21 hours in Meghalaya and 2.93 hours in Nagaland and for animal-based NTFPs was 3.62 and 4.44 hours, respectively. Weekly, respondents collected 14.17 kg and 10.04 kg of plant-based NTFPs in Meghalaya and Nagaland, respectively, and 2.66 kg and 12.30 kg of animal-based NTFPs. Bamboo shoots gave the highest annual return in Meghalaya, while wild black pepper was most valuable in Nagaland.

Keywords: NTFPs, North-east India, Meghalaya, Nagaland, Valuation

NTFPs play a significant role in providing income for the poor in many countries, with approximately 50 million people in India relying on NTFPs for subsistence and cash income (Talukdar *et al*., 2021). NTFPs are rich in biodiversity and serve as a source of food, fodder, fiber, fertilizers, herbal products, construction materials, cosmetics, and cultural products such as perfumes, medicines, and paints (Saikia et al., 2017)).Valuing the environment is crucial for sustainability, assessing management, and incorporating non-measurable environmental impacts in cost-benefit analysis (Kiran and Kaur, 2011). Methods of economic valuation consist of three procedures *viz*., (i) Revealed preference approach (ii) Stated preference approach (iii) Benefits transfer. Revealed preference uses actual market prices, while stated preference creates hypothetical markets. (Brander et al., 2024). The revealed preference approach uses market price analysis to examine changes in commodity prices in economic markets (Kiran et al., 2016).The Eastern Himalayas, also known as Purvanchal Himalayas, encompass the southern border of Arunachal Pradesh and extend in a north-south direction through Nagaland, Manipur, Mizoram, and Tripura. The region is joined by the Meghalaya Plateau in the west. Nagaland and Meghalaya are two Eastern Himalayan states of North East India that were chosen for the study because of the abundance of NTFPs and their various uses by indigenous communities. The North East region of India covers an area of 255,088 km^2, accounting for 7.7% of India's total geographical area. This region boasts diverse flora and fauna, as well as over 200 distinct ethnic groups. Many communities in this area rely on forest products, particularly NTFPs, which play a vital role in their subsistence and food security. Nagaland and Meghalaya have dense forests covering approximately 78% of their total geographical area. These forests are crucial for the livelihoods of around 5.4 million people in the region, who directly or indirectly depend on NTFPs (Kaushik and Banik 2020). The study aimed to estimate the economic value of Non-Timber Forest Products (NTFPs) available in the Eastern Himalayan states.

MATERIAL AND METHODS

The proposed study was conducted in the Eastern Himalayan states of Meghalaya and Nagaland.An exploratory research design was used and the type of data collected were panel data with a purposive sampling technique. Meghalaya and Nagaland were selected purposefully for the proposed studybecause of their rich biodiversity. Altogether, total number of 20 villages were selected. From the 20 selected villages, a total of 250 respondents were selected proportionately based on the availability of the number of households in the villages (Table 1, 2).

Table 1. Geographic and demographic features of the study sites in Meghalaya

Particulars	East Khasi Hills					Rhi-boi				
Name of village	Thangtim	Mawmang	Kongthong	War War	Kshaid	Umran	Umsning	Mawknor	Umshorshor	Umiarong
Geographic position	25.32°N ,91.81°E	25.30°N 91.83°E	25.31°N 91.83°E	25.33°N 91.79°E	25.36°N 91.77°E	25.77°N 91.87°E	25.75°N 91.89°E	25.73°N 91.87°E	25.72°N 91.85°E	25.70°N 91.87°E
Mean altitude (m)	1304	861	874	1444	631	690	774	806	770	826
Population of the village	108	183	567	213	287	1370	1176	148	269	477
Number of households	22	46	109	45	59	268	218	27	55	93
Sample size (*n*)	3	6	14	6	8	36	29	4	7	12

Table 2. Geographic and demographic features of the study sites in Nagaland

Particulars	Peren					Mokokchung				
Name of village	Kendung	Kipeujang	Mpai old	Ndunglwa	Peletkie	Mangkolemba	Japu	Satsukba	Khar	Chungliyimsen
Geographic position	25.35°N 93.69°E	25.54°N 93.77°E	25.38°N 93.70°E	25.33°N 91.79°E	25.53°N 93.75°E	26.50°N 94.44°E	26.50°N 94.41°E	26.45°N 94.35°E	26.47°N 94.47°E	26.45°N 94.42°E
Mean altitude (m)	542	816	1038	776	1382	589	619	504	763	1098
Population of the village	101	674	693	424	300	3713	487	242	3614	337
Number of households	21	125	174	106	64	879	134	65	848	103
Sample size (*n*)	3	16	22	14	8	27	4	2	26	3

The analytical framework

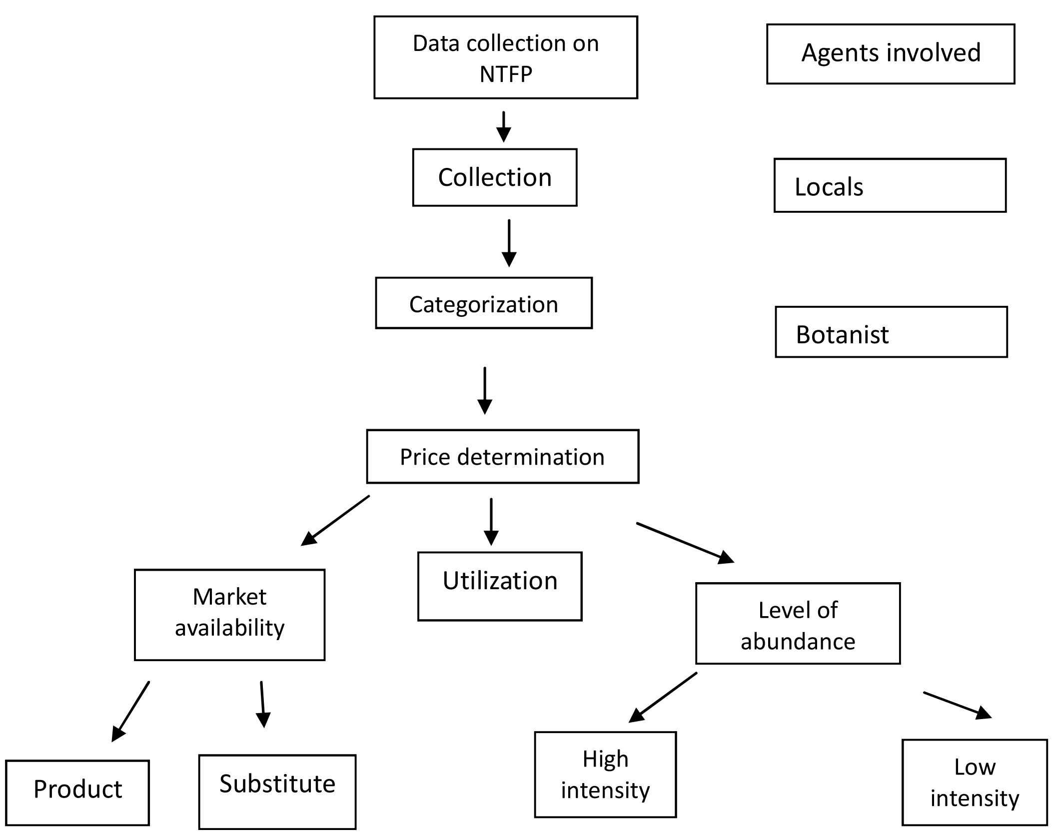

Fig. 1. Methods for valuing non-timber forest products

Figure 1 demonstrates the analytical framework for evaluating the NTFP collected in the study areas beginning with the inception of collection of the NTFP through verbal and observational data from the respondents followed by its scientific identification based on the nature and usage of the NTFP. Price validation is assigned to the NTFPs either by the prevailing market price or the price of its substitute product in the market.

Valuation: The value of NTFPs collected was estimated by using the direct use value (revealed market price) method acknowledging the cost-benefit analysis and the NTFP collected were categorized as a part of forest resources having consumptive uses. The following variables were used to supplement the valuation procedure as well as to forecast a pattern in their structure of NTFP collection.

a) NTFP (animal-based or plant-based)

b) Parts used

c) Season of collection (summer, winter or year-round)

d) Collection trip per week

e) Average time spent (hrs) per trip

f) Quantity collected per trip(kg)

g) Distance travelled to collect (km)

h) Existing Market price (₹kg^{-1})

i) Monetary value (₹$year^{-1}$)

RESULTS AND DISCUSSION

Identification and classification of NTFPs: In total 47 NTFPs were identified and categorized based on their uses such as food, medicinal supplements, decorative items, construction materials, and fuel wood. The identification and categorization of the different non-timber flora and fauna found in Meghalaya presented in Table 3. The majority of 17 (77.27%) were plant-based products, whereas 5 (27.73%) were derived from animals. Among the NTFPs gathered, the predominant uses were for nourishment (59.09%), therapeutic purposes (22.73%), housing materials (13.64%), and fuel wood (4.55%).

Tribal of Nagaland use traditional indigenous knowledge of NTFPs for nutrition and medicine, passed down orally through generations (Walling et al., 2021). Altogether 16 plant-based and 9 animal-based products were identified. These NTFPs were gathered from the region, reflecting the diverse range of uses attributed to them. The majority of the collected NTFPs in Nagaland were primarily utilized for nourishment purposes, accounting for 60.00% followed by therapeutic properties (24.00%), as housing materials (12.00%), as fuel wood (4%). Further classification based on the nature of the NTFPs revealed that the majority were plant-based, 64.00% of the total. This classification highlights the significant reliance on plant-derived resources among the indigenous tribes of Nagaland for various purposes, including nutrition, medicine, construction, and fuel.

The people of Meghalaya rely on a variety of non-timber forest products (NTFPs) for their food, medicine, and utility needs (Table 5, 6). Plant-based NTFPs include edible fruits like Burmese grape (*Baccuarea sapida*), Indian plum (*Flacourtia jangomas*), and bayberry (*Myrica esculenta*), as well as medicinal plants such as Indian gooseberry (*Phyllanthus emblica*). Other plant parts like banana buds, bamboo shoots, and tender fern leaves are utilized as food sources, while materials like thatch leaves and bamboo stems serve as roofing and construction materials. The animal-based NTFPs collected include honey from the Asian honeybee (*Apis dorsata*), shrimp, and meat from animals like flying lizards and common carp, with some also used for medicinal purposes.

The people of Nagaland similarly gather a wide range of NTFPs for food, medicine, and functional uses. Plant-based products include leaves of colocasia (*Colocasia sp.*) and ketibu (*Uroticadiocia*), fruits such as myrobalan (*Terminalia chebula*) and Chinese sumac (*Rhus chinenses*), and construction materials like bamboo stems. Medicinal plants like Indian gooseberry (*Phyllanthus emblica*) and Indian nightshade (*Solanum violaceum*) are also utilized for their health benefits. On the animal side, honey from the Asian honeybee (*Apis dorsata*), meat from wild pigs (*Sus scrofa cristatus*), and decorative items like porcupine quills are collected.

Economic importance: NTFPs products like Kanthior (*Sterculia roxburghii*), Ferns (*Dryopten* ssp.), Bamboo (*Bambusa* sp.), and Pine wood (*Pinus* spp.) were available year-round while important NTFPs like Banana buds (*Bambusa* sp.), Bayberry (*Myrica esculenta*), Mushroom (*Pleurotus*sp.) and Burmese grape (*Baccuarea sapida*) were available during summer and NTFPs such as Indian plum (*Flacourtia jangomas*), Indian gooseberry (*Phyllanthus Emblica* L.), Wild apple (*Docynia indica*) and bay leaves (*Cinnamomum tamala*) were available during the winter season (Table 5). Households went on varying numbers of trips for the collection of Non-Timber Forest Products (NTFPs), depending on the type of NTFP and its use. In most of the cases respondents went out 2-3 times per week for NTFPs collection, except for banana buds and thatch leaves, which required 4 trips per week. In the case of brown shrimp, common carp fish, and flying lizard, only one trip was made.The average time spent per trip for plant-based NTFPs was 2.21 hours. The maximum time was dedicated for collecting bamboo shoots (4.16 hours), banana buds (3.56 hours), and bag flower (3.15 hours). Regarding animal-

Table 3. Identification and categorization of Non-Timber Forest Products found in Meghalaya

NTFPs categorization		No products/species
Nourishments	Plant-based	10
	Animal-based	3
	Subtotal	13 (59.09)
Therapeutic	Plant-based	3
	Animal-based	2
	Subtotal	5 (22.73)
Housing material	Plant-based	3
	Animal-based	0
	Subtotal	3 (13.64)
Fuelwood	Plant-based	1
	Animal-based	0
	Subtotal	1 (4.55)
Plant-based NTFPs		17 (77.27)
Animal-based NTFPs		5 (27.73)

Figures in parentheses represent percentage to total

Table 4. Identification and categorization of non-timber Forest Products found in Nagaland

NTFPs categorization		No products/species
Nourishments	Plant-based	7
	Animal-based	8
	Sub total	15 (60.00)
Therapeutic	Plant-based	5
	Animal-based	1
	Sub total	6 (24.00)
Housing material	Plant-based	3
	Animal-based	0
	Sub total	3 (12.00)
Fuel-wood	Plant-based	1
	Animal-based	0
	Sub total	1 (4.00)
Plant-based NTFPs		16 (64.00)
Animal-based NTFPs		9 (36.00)

Figures in parentheses represent percentage to total

Table 5. Non-timber forest products collected by the people of Meghalaya state

Plant-based				Animal-based			
Common name	Scientific name	Part collected	Use	Common name	Scientific name	Part collected	Use
Burmese grape	*Baccuarea sapida*	Fruit	Food	Asian honeybee	*Apisdorsata*	Honey	Food/medicine
Bag flower	*Clerodendrum colebrookianum*	Tender leaves	Food/medicine	Brown shrimp	*Crangon crangon*	Meat	Food
Giant Indian fig	*Ficus roxburghii*	Tender leaves/fruit	Food	Common carp	*Cyprinus carpio*	Meat	Food
Indian plum	*Flacourtia jangomas*	Fruits	Food	Flying lizard	*Draco norvilli*	Meat	Medicine
Banana buds	*Musa spp.*	Buds	Food	Mushroom	*Pleurotus* sp.	Whole part	Food/medicine
Bayberry	*Myrica esculenta*	Fruits	Food/medicine				
Indian gooseberry	*Phyllanthus Emblica L.*	Fruit	Medicine				
Kanthior	*Sterculia roxburghii*	Fruits	Food				
Broom grass	*Thysanolaena maxima*	Anthesis, stem	Utility				
Bamboo shoot	*Bambusa*sp.	Tender shoots	Food				
Ferns	*Dryoptens*sp.	Tender leaves	Food				
Thatch leaf	*Calamus arboresence*	Leaves	Roofing material				
Bamboo	*Bambusa* sp.	Stem	Roofing material/ construction				
Pinewood	*Pinus* sp.	Whole plant/resins	Fuelwood/ornate				
Wild apple	*Docynia indica*	Fruit	Food/medicine				
Betel leaf vine	*Piper bettle*	Leaves	Tobacco				
Bay leaves	*Cinnamomum Tamala*	Leaves	Food/medicine				

based NTFPs, the average time spent per trip for collection was 4.02 hours, with the highest time spent on collecting common carp fish (4.38 hours), followed by flying lizard (4.02 hours).The distance travelled by households for NTFP collection was categorized into three groups: less than 2 km, between 2-5 km, and more than 5 km. For plant-based NTFPs like bag flower, banana buds, and broom grass, households travelled more than 5 km. For NTFPs such as Indian plum, bayberry, and thatch leaves, the distance travelled was between 2-5 km. For Burmese grape, kanthior, and wild apple, the distance travelled was less than 2 km. Regarding animal-based NTFPs like Asian honey bee, common carp fish, brown shrimp, and mushroom, villagers travelled more than 5 km.

Important NTFPs products which were available year-round were colocasia (*Colocasia*sp.), Pinewood (*Pinus* sp.), Bamboo (*Bambusa* sp.), bamboo shoot (*Bambusa* sp.), Asian honeybee (Apisdorsata), chocolate Mahseer (*Neolissochilushexagonolepis*), wild pig (*Sus scrofa cristatus*), jungle fowl (*Gallus gallus*), mushroom (*Pleurotuspetaloides*) and snail(*Pila spp*.), while NTFPs like myrobalan (*Terminalia chebula* Retz.), Indian nightshade (*Solanum violaceum*), chinese sumac (*Rhus chinenses*), and tree bean (*Parkia roxburghii*) were available during the summer season and NTFPs like Indian gooseberry (*Phyllanthus Emblica* L.), ferns (*Dryoptens*sp.), wild black pepper (*Piper* sp.) and broom grass (*Thysanolaena maxima*) were available during the winter season. The average trip per week for collecting NTFPs recorded highest (5 nos.) in bamboo shoot followed by bamboo (4 nos.), 3 times in a week in case of wild black pepper, tree bean and broom grass, etc., twice in a week in case of Colocasia, Chinese sumac and Asian honey bee, etc., while NTFPs like Indian gooseberry, Indian nightshade, and jungle fowl etc. were collected only once in a week. The average time spent per trip for plant-based NTFPs recorded as 3.33 hours where the maximum time was spent in the collection of bamboo (5.18 hrs), broom grass (4.37 hrs), and bamboo shoot (4.34 hrs). For animal-based NTFPs, the average time spent per trip was 4.44 hours. Maximum time was spent in the collection of NTFPs

Table 6. Non-timber forest products collected by the people of Nagaland state

Plant-based				Animal-based			
Common name	Scientific name	Part collected	Use	Common name	Scientific name	Part collected	Use
Colocasia	*Colocasia* sp.	Leaves	Food	Asian honeybee	*Apisdorsata*	Honey	Food/medicine
Myrobalan	*Terminalia chebula* Retz.	Fruits	Food /medicine	Chocolate Mahseer	*Neolissochilus hexagonolepis*	Meat	Food
Indian gooseberry	*Phyllanthus Emblica* L.	Fruit	Medicine	Tsuma Ngai (fish)	-		
Ferns	*Dryoptens* sp.	Tender leaves	Food	Wild pig	*Sus scrofa cristatus*	Meat	Food/ornaments
Wild black pepper	*Piper* sp.	Fruit	Food/medicine	Jungle fowl	*Gallus*	Meat/feathers	Food/ornaments
Pinewood	*Pinus* sp.	Whole plant/resins	Fuelwood/ornate	Frog	Varied	Meat	Food
Ketibu	*Uroticadiocia*	Leaves	Food/medicine	Porcupine	*Hystrix indica*	Meat/quills	Food/ornaments
Indian nightshade	*Solanum violaceum*	Roots/fruits	Food/medicine	Mushroom	*Pleurotuspetaloides*	Whole part	Food/medicine
Chinese sumac	*Rhus chinenses*	Fruit	Food/medicine	Snail	*Pila* sp.	Fleshy innards	Food/medicine
Common walnut	*Juglans regia*	Seed	Food				
Bamboo	*Bambusa*sp.	Stem	Roofing material /construction/ornate				
Wild apple	*Docynia indica*	Fruit	Food/medicine				
Toko patta	*Livistona jenkensiana*	Berry/branch	Food/roofing material				
Tree bean	*Parkia roxburghii*	Pod	Food				
Broom grass	*Thysanolaena maxima*	Anthesis, stem	Utility				
Betel leaf vine	*Piper bettle*	Leaves	Tobacco				

like porcupine (6.07 hrs), jungle fowl (5.38 hrs) and wild pig (5.27 hrs). NTFPs such as chocolate mahseer, wild pig, and porcupine, etc. respondents travelled more than 5 km for their collection, while jungle fowl, frog and mushroom were collected after travelling a distance between 2-5 km.

The average quantity of plant-based NTFPs collected in a week from Meghalaya recorded as 14.17 kg, where maximum quantity of NTFPs collected were bay leaves (70.38 kg) followed by bamboo shoot (12.00 kg) and banana buds (11.00 kg). The average quantity of animal-based NTFPs collected in a week from Meghalaya was 2.66 kg, where among the animal-based NTFPs, mushroom (6.00 kg) and Asian honey bee (2.00 kg) were collected in maximum quantities. Among the NTFPs collected in the State, NTFPs *viz*. bamboo shoot gave the highest value of return (₹50406 household^{-1}), followed by bay leaves (₹40533year^{-1}) and pine wood (₹33682 year^{-1}) and these were some of the highly sought NTFPs with high value compared to the other NTFPs (Table 9).

The average weekly collection of plant-based NTFPs was 10.04 kg, with bamboo shoot being the most abundant at 32.40 kg, followed by wild apple at 23.56 kg, and Indian gooseberry at 10.22 kg. As for animal-based NTFPs, the average weekly collection amounted to 12.30 kg. Among the

Asian honeybee larva *(Apisdorsata)*

Mushroom (*Agaricus bisporus)*

Snail *(Pila* sp.*)*

Jungle fowl (*Gallus gallus)*

Tsuma Ngai (fish) scientific name (NA)

Wild pig (*Sus scrofa cristatus)*

Fig. 3a. Animal-based NTFPs

Table 7. Season of collection, collection trip per week, average time spent (hrs) per trip and distance (km) travelled for NTFPs collection in Meghalaya State

NTFPs	Parts used	Season of collection	Collection trip per week	Average time spent (hrs) per trip	Distance travelled to collect (km)
Burmese grape	Fruit	Summer	2	2.18	<2
Bag flower	Tender leaves	Winter	3	3.15	>5
Giant Indian fig	Tender leaves/fruit	Winter	2	1.50	<2
Indian plum	Fruits	Winter	3	2.00	2~5
Banana buds	Buds	Summer	4	3.56	>5
Bayberry	Fruits	Summer	2	1.34	2~5
Indian gooseberry	Fruit	Winter	3	1.29	>5
Kanthior	Fruits	Year-round	3	1.07	<2
Broom grass	Anthesis, stem	Winter	2	3.05	>5
Bamboo shoot	Tender shoots	Summer	3	4.16	>5
Ferns	Tender leaves	Year-round	2	1.49	2~5
Thatch leaf	Leaves	Summer	4	2.45	2~5
Bamboo	Stem	Year-round	3	2	2~5
Pinewood	Whole plant/resins	Year-round	3	2.18	2~5
Wild apple	Fruit	Winter	2	1.46	<2
Bay leaves	Leaves	Winter	3	2.45	>5
Asian honeybee	Honey	Year-round	2	3.29	>5
Brown shrimp	Meat	Year-round	1	3.16	>5
Common carp	Meat	Year-round	1	4.38	>5
Flying lizard	Meat	Year-round	1	4.02	<2
Mushroom	Whole part	Summer	3	3.27	>5

animal-based NTFPs collected, the highest quantities were attributed to wild pig at 43.34 kg and mushroom at 15.32 kg.Based on their existing market prices, the NTFPs with high value were identified. Wild black pepper ranked first with a monetary value of ₹134,306.10 year^{-1}, followed by chocolate masheer at ₹87,460 year^{-1}, and Asian honeybee at ₹70,180year^{-1}. Furthermore, the average weekly collection of animal-based NTFPs reached 10.64 kg (Table 10).

The total value of the NTFPs collected annum^{-1}from Meghalaya was ₹433455, whereas plant-based NTFPs was ₹330689 and the animal-based NTFPs was ₹102766 (Table 11). The total value of the NTFPs collected annum^{-1}from Nagaland was ₹980991, whereas the value of the plant-based NTFPs was ₹523242 and the animal-based NTFPs was ₹457749.

From the NTFPs collected in Meghalaya, the monetary value of NTFPs seemed highest which were available year-round (₹153053 annum^{-1}) followed by winter season (₹141106.12 annum^{-1}) and summer (₹139295.56annum^{-1}) (Table 11). The monetary value of NTFPs which were available year-round (₹585584.79annum^{-1}) was the highest followed by NTFPs which available during the winter season (₹263831annum^{-1}) and summer (₹131575 annum^{-1}).The monetary value ofplant-based NTFPs collected from Meghalaya was highest in case of NTFPs available during winter (₹141106.12 annum^{-1}) followed by summer (₹108026 annum^{-1}) and year-round (₹81556 annum^{-1}). The monetary value of animal-based NTFPs collected from Meghalaya was highest which were available during year-round (₹71497) followed by animal-based NTFPs available during summer (₹31268 annum^{-1}). The plant-based NTFPs collected from Nagaland was highest which were available during winter

Table 8. Season of collection, collection trip per week, average time spent (hrs) per trip and distance (km) travelled for NTFPs collection in Nagaland State

NTFPs	Parts used	Season of collection	Collection trip per week	Average time spent (hrs) per trip	Distance travelled to collect (km)
Colocasia	Leaves/corms	Year-round	2	2.31	>5
Myrobalan	Fruits	Summer	2	2.24	<2
Indian gooseberry	Fruit	Winter	1	1.23	>5
Ferns	Tender leaves	Winter	2	3.45	>5
Wild black pepper	Fruit	Winter	3	3.38	>5
Pinewood	Whole plant/resins	Year-round	2	2.23	2-5
Ketibu	Leaves	Winter	1	2.3	2-5
Indian nightshade	Roots/fruits	Summer	1	1.22	2-5
Chinese sumac	Fruit	Summer	2	1.43	<2
Common walnut	Seed	Summer	2	3.27	2-5
Bamboo	Stem	Year-round	4	5.18	2-5
Bamboo shoot	Tender shoots	Year-round	5	4.34	>5
Wild apple	Fruit	Winter	2	2.65	>5
Toko patta	Berry/branch	Winter	3	4.05	>5
Tree bean	Pod	Summer	3	3.23	2-5
Broom grass	Anthesis, stem	Winter	3	4.37	2-5
Asian honeybee	Larvae/honey	Year-round	2	4.29	2-5
Chocolate Mahseer	Meat	Year-round	2	3.33	>5
Tsumangai (fish)	Meat	Year-round	2	4.01	>5
Wild pig	Meat/ tusks, teeth	Year-round	1	5.27	>5
Jungle fowl	Meat/feathers	Year-round	1	5.38	2-5
Frog	Meat	Year-round	2	3.45	2-5
Porcupine	Meat/quills	Year-round	1	6.07	>5
Mushroom	Whole part	Year-round	2	4.16	2-5
Snail	Fleshy innards	Year-round	2	4.01	>5

Table 9. Quantity collected per trip (kg), market price and monetary value (₹year^{-1}) of NTFPs collected in Meghalaya

NTFPs	Parts used	Quantity week^{-1} (kg)	Existing market price (₹kg^{-1})	Monetary value (₹year^{-1})
Burmese grape	Fruit	5.00	21.86	5683
Bag flower	Tender leaves	6.00	36.45	11372
Giant Indian fig	Tender leaves/fruit	3.00	27.32	4261
Indian plum	Fruits	6.00	91.10	28423
Banana buds	Buds	11.00	45.56	26060
Bayberry	Fruits	1.60	95.56	7950
Indian gooseberry	Fruit	9.00	60.95	28524
Kanthior	Fruits	6.00	27.32	8523
Broom grass	Anthesis, stem	8.00	45.56	18952
Bamboo shoot	Tender shoots	12.00	80.78	50406
Ferns	Tender leaves	2.34	58.57	7126
Thatch leaf	Leaves	17.00	20.22	17925
Bamboo	Stem	60.00	24.00	32223
Pine wood	Whole plant/resins	5.40	120.00	33682
Wild apple	Fruit	4.00	43.45	9037
Bay leaves	Leaves	70.38	33.34	40533
Asian honeybee	Honey	2.00	273.34	28427
Brown shrimp	Meat	1.50	91.11	7106
Common carp	Meat	2.00	265.32	27593
Flying lizard	Meat	1.78	90.43	8370.20
Mushroom	Whole part	6.00	100.22	31268

Table 10. Quantity collected per trip (kg), market price and monetary value (₹year^{-1}) of NTFPs collected in Nagaland state

NTFPs	Parts used	Quantity per week (kg)	Existing market price (₹ kg^{-1})	Monetary value (₹year^{-1})
Colocasia	Leaves/corms	6.23	61.80	26423
Myrobalan	Fruits	8.20	90.60	48280
Indian gooseberry	Fruit	10.22	56.45	34026
Ferns	Tender leaves	5.78	136.66	21377
Wild black pepper	Fruit	3.00	386.36	134306
Pinewood	Whole plant/resins	10.00	95.78	34478
Ketibu	Leaves	7.20	47.89	25470
Indian nightshade	Roots/fruits	5.10	24.87	10374
Chinese sumac	Fruit	4.00	50.00	21378
Common walnut	Seed	8.20	92.57	13515
Bamboo	Stem	2.80	22.34	18052
Bamboo shoot	Tender shoots	32.40	114.28	56156
Wild apple	Fruit	23.56	45.76	10021
Toko patta	Berry/branch	4.20	10.85	2010
Tree bean	Pod	5.33	122.96	35605
Broom grass	Anthesis, stem	8.33	28.45	31764
Asian honeybee	Larvae/honey	22.33	405.67	70180
Chocolate Mahseer	Meat	3.46	296.98	87460
*Tsumanga*i (fish)	Meat	5.89	126.97	40757
Wild pig	Meat/ tusks, teeth	43.34	689.34	56961
Jungle fowl	Meat/feathers	6.42	476.21	54630
Frog	Meat	4.78	87.90	24392
Porcupine	Meat/quills	5.55	543.65	19988
Mushroom	Whole part	15.32	212.76	37977
Snail	Fleshy innards	3.57	176.52	65400

Table 11. Monetary value of NTFPs collected based on their nature and season of collection

Particulars		Monetary value ₹ year^{-1}	
		Meghalaya	Nagaland
Nature			
Plant-based		330689	523242
Animal-based		102766	457749
Total		433455	980991
Season of collection			
Summer		139295	131575
Winter		141106	263831
Year-round		153053	585584
Total		433455	980991
Season of collection and types of NTFPs collected			
Summer	Plant-based	108026	129153
	Animal-based	31268	0.00
Winter	Plant-based	141106	258976
	Animal-based	0.00	0.
Year-round	Plant-based	81556	135111
	Animal-based	71497	457749
Total		433455	980991

Bamboo shoot (*Bambusa* sp.)

Giant Indian fig(*Ficus roxburghii)*

Colocasia(*Colocasia* sp.)

Common walnut(*Juglans regia)*

Banana buds*(Musa* sp.)

Tree bean *(Parkia roxburghii)*

Ferns (*Dryoptens* sp.)

Fig. 3b. Plant-based NTFPs (nourishment)

Wild gooseberry (*Phyllanthus Emblica* L.)

Bag flower (*Clerodendrum colebrookianum)*

Chinese sumac*(Rhus chinenses)*

Indian nightshade (*Solanum violaceum)*

Wild apple(*Docynia indica)*

Wild black pepper (*Piper* sp.)

Bayberry (*Myrica esculenta*)

Fig. 3c. Plant-based NTFPs (therapeutic)

(₹258976 annum^{-1}) followed by year-round (₹135111annum^{-1}) and summer (₹129153 annum^{-1}). The animal-based NTFPs collected from Nagaland were available all year-round with a monetary value of ₹457749 annum^{-1} A similar study by Yadav et al. (2020) conducted on the monetary value of plant-based and animal-based NTFPs in Valsad North and South Forest Divisions (VNFD and VSFD) were similarly evaluated. VNFD and VSFD collectively housed 23 vegetable NTFPs and 1 animal NTFP, with a total quantity of 10643610.6 kg annum^{-1} and 7581813 bundles annum^{1} of *Diospyros melanoxylon* leaves. The monetary value of these NTFPs was assessed at ₹230.40 lakhs annum^{1}, contributing significantly to the local economy. The study underscores how NTFP diversity and economic benefits impact the livelihoods and subsistence of forest dwellers in these regions.

CONCLUSION

Non-Timber Forest Products (NTFPs) play a vital role in the Eastern Himalayan region of India, supporting biodiversity conservation, rural livelihoods, local economies, cultural heritage, and traditional healthcare systems. They are collected for subsistence and cultural purposes, extending beyond market value. NTFPs provide employment and entrepreneurship opportunities, especially for marginalized communities in remote areas, reducing reliance on traditional agriculture. Many NTFPs possess medicinal properties, contributing to herbal remedies. Sustainable management, including ecological assessments and harvesting guidelines, is essential to ensure future availability. Valuing NTFPs involves considering their market prices, ecological services, subsistence importance, and cultural significance, showcasing their immense contribution to the region's economy and society.

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Floristic Diversity and Biological Spectrum in Urban and Peri-urban Areas of Ganderbal, Kashmir

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Abstract: The study was conducted to investigate tree diversity, life forms and leaf spectra during 2022 and 2023.Vegetation analysis was conducted in nine wards of Municipal Committee of Ganderbal and peri-urban locations within a 1-2 km vicinity of the wards. Random sampling of trees, shrubs and herbs was carried out using 10 m x 10 m, 5 m x 5 m and 1 m x 1 m quadrants, respectively. The study identified 115 species across 50 families, comprising 101 dicots, 8 monocots and 6 gymnosperms. Dominant families included Asteraceae (11.30 %), Rosaceae (9.56 %), Leguminosae (6.08 %) and Amaranthaceae (5.21 %). *Prunus* and *Rosa* were the most diverse genera, each with three species. Moreover, two species were recognized as endemic, with an additional 12 species classified as native to the study area. There was greater biodiversity in urban regions than in peri-urban areas, highlighting urbanisation's impact on plant richness and composition. Of the surveyed species, trees, shrubs and herbs were 225.22 , 19.13 and 51.30 % respectively emphasizing the prevalence of herbaceous vegetation. According to Raunkiaer's life form system, therophytes (34.78 %) were predominant, followed by nanophanerophytes (17.39 %), indicating a thero-phanerophytic phytoclimate. Microphylls (34.78 %) and simple leaves (66.09 %) were prevalent in the leaf size spectrum and leaf lamina type. The dominance of therophytes suggests an adaptation to harsh climate conditions and anthropogenic pressures in urban and peri-urban areas.

Keywords: Floristic diversity, Biological spectrum, Urban and Peri-urban

Floristic diversity and biological spectrum have been extensively studied by researchers worldwide. Numerous studies have examined the floristic composition and biological spectrum of forests across various regions, significant knowledge gaps persist, particularly in the Kashmir Himalayas. Wani and Pant (2023) explored the floristic diversity and community characteristics of Gulmarg Wildlife Sanctuary in the Kashmir Himalaya, while Pulicherla et al. (2023) conducted a floristic exploration of the East Sikkim district in Sikkim. Haq et al. (2023) provided a comprehensive analysis of vegetation composition in the Shiwalik Mountain Range Forest. Similarly, Surmal et al. (2022) investigated species diversity in the coniferous forests of Bhallesa Hills in the Pir Panjal Mountain range of the Western Himalaya. Further, Rahman et al. (2021) examined the biological spectrum of vascular flora in Zaini Pass, District Chitral, Pakistan, whereas Sen and Bhakat (2020) conducted a quantitative analysis of floristic composition, biological spectrum, and leaf spectrum in a sacred grove in Jhargram District, West Bengal.. Despite these contributions, the lack of research in urban environments necessitates focused efforts to better understand the floristic diversity and biological spectrum within these evolving landscapes.

MATERIAL AND METHODS

Study area: The investigation was conducted in Ganderbal district, Jammu and Kashmir UT,during 2022-2023.Ganderbal is located between 34.23°N and 74.78°E at an elevation of 1650 to 3000 meters above sea level. The district, situated on the left bank of River Sindh, covers 39,304 hectares, comprising forest (27.86 %), non-agricultural use (14.65 %), barren and un-cultivable land (8.04 %), permanent pastures / other grazing land (4.55 %), cultivable waste land (2.48 %) and net area sown (42.42 %) (Anonymous 2011).

Data collection: The floristic diversity and phytosociological characteristics of vegetation in urban and peri-urban areas of Ganderbal were conducted through field surveys in nine wards of the Municipal Committee of Ganderbal and nine peri-urban areas within 1-2 km of the wards. Using multistage random sampling, 630 quadrants were laid, with 5 for trees, 10 for shrubs and 20 for herbs per location. Trees were sampled using species-area curve-determined quadrats, while shrubs and herbs were sampled with 5m x 5m and 1m x 1m quadrats, respectively. Standard ecological methods were followed for data collection (Curtis and McIntosh 1950, Greig-Smith 1957, Misra 1968, Mueller-Dombois and Ellenberg 1974). The similarity index was determined using the Sorenson index, while the diversity index was calculated according to the Shannon-Wiener diversity function (Shannon-Wiener 1963).

Biological spectrum: In-depth field observations on aspects like habit (growing form, Raunkiaer's life form), leaf type (size, lamina) and other characteristics of each species were observed. Growth forms were divided into tree, shrub, sub-shrub, herb, palm and climberbased on growth direction, size and main shoot branching (Perez-Harguindeguy et al., 2013). Life forms were categorized by the location of the perennating bud during unfavourable seasons into geophytes, therophytes, hemicryptophytes, chamaephytes, nanophanerophytes, microphanerophytes, mesophanerophytes and megaphanerophytes (Muzafar et al., 2019). Leaf size, indicative of local edaphic and climatic conditions, was classified into leptophyll, nanophyll, microphyll, mesophyll, macrophyll and megaphyll (Ali et al., 2016). Leaf lamina shapes were as simple, dissected, compound and needle, reflecting adaptations for optimal light capture (Malhado et al., 2009).

RESULTS AND DISCUSSION

Floristic diversity: The investigation unveiled 115 plant species representing 102 genera across 29 plant orders belonging to 50 plant families (Table 1). This included 8 species as monocotyledons, 6 species as gymnosperms and 101 species as dicotyledonous. Asteraceae emerged as the most diverse family with 13 species, followed by Rosaceae with 11 species. Leguminosae and Amaranthaceae encompassing 7 and 6 species, respectively. Verma and Kapoor(2011) highlighted Asteraceae, Rosaceae and other families as dominant .The prevalence of Asteraceae can be attributed to their adaptive seed dispersal mechanisms, ecological tolerance and ability to thrive across various environmental conditions (Awas and Demissew, 2009). The diverse genera in terms of species richness were *Prunus* and *Rosa*, each comprising three species (Fig. 1). The two species were identified as endemic; while 12 species were categorized as native to the study area (Fig. 2). Khuroo et al. (2017) reported a higher proportion of alien species (52 %) compared to native species (48 %) in an urban biodiversity hotspot in the Indian Himalayas. The prevalence of non-native species along roadsides is likely due to frequent anthropogenic disturbances, such as fragmentation, pollution, and the heat island effect, which create favourable conditions for their establishment. Trombulak and Frissell (2000) and Lippe and Kowarik (2008) observed that T species from families Asteraceae and Poaceae often become dominant invaders in urban areas .In present study in urban area there were 27 tree species, 21 shrubs, 59 herbs, 2 sub-shrubs and 2 climbers, while peri-urban areas had 17 tree species, 16 shrubs, 43 herbs and 1 sub-shrub (Fig. 3).

Shannon-Wiener Index (H') of urban and peri-urban

Table 1. Plant species in the urban and peri-urban areas of ganderbal

Name	Family	Common name
Abelia × grandiflora Rehder	Caprifoliaceae	Glossy Abelia
Aesculus indica (Wall. ExComb.) Hk. f.	Sapindaceae	Indian horse Chestnut
Ailanthus altissima Swingle.	Simoaroubaceae	Tree of Heaven
Alcea rosea L.	Malvaceae	Hollyhock
Amaranthus caudatus L.	Amaranthaceae	Velvet flower
Amaranthus tricolor L.	Amaranthaceae	Edible amaranth
Anthemis cotula L.	Asteraceae	Sticking Chamomile
Antirrhinum majus L	Plantaginaceae	Dog Flower
Arctium lappa L.	Asteraceae	Greater Burdock
Avena sativa L.	Poaceae	Oat
Berberis lycium Royle	Berberidaceae	Indian Barberry
Bothriochloa ischaemum L.	Poaceae	Yellow bluestem
Brassica rapa L.	Brassicaceae	Field mustard
Buddleja davidii Franch.	Scrophulariaceae	Butterfly brush
Buxus sempervirens L.	Buxaceae	Boxwood
Callistephus chinensis (L.) Nees	Asteraceae	Annual Aster
Campsis grandiflora (Thunb.) K. Schum.	Bignonaceae	Trumpet
Canna indica L.	Cannaceae	Indian shot
Cannabis sativa L.	Cannabaceae	Marijuana
Carpesium abrotanoides L.	Asteraceae	Carpesium Fruit
Catalpa bignoniodes Walter	Bignoniaceae	Indian Bean Tree
Cedrus deodara (Roxb.)	Pinaceae	Deodar
Celosia argentea L.	Amaranthaceae	Plumed Cock's Comb
Celtis australis L.	Cannabaceae	European nettle tree
Cercis siliquastrum L.	Leguminosea	Judas Tree
Chenopodium album L.	Amaranthaceae	White goosefoot
Cirsium arvense Scop.	Amaranthaceae	Wool bearing Thistle
Clarkia pulchella Pursh	Onagraceae	Ragged Robin
Convolvulus arvense L.	Convolvaceae	Field Bindweed
Conyza canadensis L.	Asteraceae	Horseweed
Crataegus songarica K.Koch	Rosaceae	Hawthorn
Cryptomeria japonica D. Don	Cupressaceae	Japanese Cedar
Cupressus sempervirens L.	Cupressaceae	Meditteranean cypress
Dahlia pinnata Cav.	Asteraceae	Garden dahlia
Daucus carota L.	Apiaceae	Wild Carrot
Dianthus caryophyllus L.	Caryophyllaceae	Clove pink
Dianthus chinensis L.	Caryophyllaceae	Rainbow pink
Eschscholzia californica Cham.	Papaveraceae	California Poppy
Euonymus japonicus Thunb.	Celastraceae	Japanese spindle

Cont...

Table 1. Plant species in the urban and peri-urban areas of ganderbal

Name	Family	Common name
Euphorbia peplus L.	Euphorbiaceae	Milkweed
Ficus carica L.	Moraceae	Common Fig
Foeniculum vulgare Mill.	Apiaceae	Fennel
Forsythia viridissima Lind.	Oleaceae	Chinese golden bell tree
Fragaria nubicola (Hoof.f) Linn.	Rosaceae	Himalayan Strawberry
Galinsoga parviflora Cav.	Asteraceae	Quick weed
Gomphrena globosa L.	Amaranthaceae	Bachelor's Button
Halianthus annus L	Asteraceae	Common sunflower
Hedera helix L.	Araliaceae	Common ivy
Hibiscus syriacus L.	Malvaceae	Rose of Sharon
Hydrangea macrophylla (Thunb.) Seringe	Hydrangeacea	French hydrangea
Hypericum androsaemum L.	Hypericaceae	St John's Wort
Iberis amara L.	Brassicaceae	Rocket Candytuft
Indigofera heterantha Wall	Leguminosae	Himalayan indigo
Juglans regia L.	Juglandaceae	English Walnut
Juniperus communis L.	Cupressaceae	Common juniper
Lagerstroemia indica L.	Lythraceae	Crape Myrtle
Lavandula angustifolia Mill.	Lamiaceae	English lavender
Lepidium sativum L.	Brassicaceae	Garden cress
Ligustrum lucidum Ait.f.	Oleaceae	Chinese privet
Lupinus polyphyllus Lindl.	Leguminosae	Garden lupin
Magnolia kobus DC	Magnoliaceae	Mokryeon
Magnolia liliflora Desr.	Magnoliaceae	Japanese
Malva neglecta Wallr.	Malvaceae	Common Mallow
Marrubium vulgare L.	Lamiaceae	Horehound
Matricaria aurea (Loefl.) Sch.Bip.	Asteraceae	Golden Chamomille
Melia azedarach L.	Meliaceae	Chinaberry tree
Mentha arvensis L.	Lamiaceae	Field Mint
Morus alba L.	Moraceae	Mulberry
Myosotis arvensis (L.) Hill	Boraginaceae	Field forget-me-not
Nerium indicum Mill.	Apocynaceae	Nerium
Oxalis corniculata L.	Oxalidaceae	Sleeping beauty
Paparver dubium L.	Papaveraceae	Long headed Poppy
Phlox drummondii Hook.	Polemoniaceae	Annual phlox
Picea smithiana (Wall.) Boiss.	Pinaceae	Western Himalayan Spruce
Plantago major L.	Plantaginaceae	Broad leaf Plantain
Platanus orientalis L.	Platanaceae	Chinar
Polygonum hydropiper L.	Polygonaceae	Water pepper
Populus nigra L.	Salicaceae	Black poplar

Cont...

Table 1. Plant species in the urban and peri-urban areas of ganderbal

Name	Family	Common name
Populus deltoides W.Bartram ex Marshall	Salicaceae	Eastern cottonwood
Prunus avium L.	Rosaceae	Wild cherry
Prunus cerasifera Ehrh.	Rosaceae	Cherry plum
Prunus persica Batsch	Rosaceae	Peach
Robinia psuedoacacia L.	Leguminosae	Black Locust
Rosa × damascena Mill	Rosaceae	Turkish rose
Rosa moschata Herrm.	Rosaceae	Musk rose
Rosa multiflora Thunb.	Rosaceae	Baby rose
Rubus niveus Thunb.	Rosaceae	Mysore Raspberry
Rubus ulmifolius Schott	Rosaceae	Elm leaf blackberry
Rumex dentatus L.	Polygonaceae	Aegean dock
Rumex hastatus D. Don	Polygonaceae	Arrowleaf Dock
Salix alba L.	Salicaceae	Willow
Salix babylonica L.	Salicaceae	Weeping Willow
Scilla siberica Andrews	Asparagaceae	Siberian squill
Setaria virdis (L.) P.Beauv.	Poaceae	Green foxtail
Sophora japonica L.	Leguminosae	Japanese pagoda tree
Spiraea japonica L.f.	Rosaceae	Japanese spiraea
Stellaria media L.	Caryophyllaceae	Chickweed
Tagetes minuta L.	Asteraceae	Mint marigold
Tagetes patula L.	Asteraceae	French marigold
Thuja orientalis L.	Cupressaceae	White cedar
Trachycarpus fortunei (Hook.) H.Wendl.	Arecaceae	Chinese windmill palm
Trifolium pratense L.	Leguminosea	Red Clover
Trifolium repens L	Leguminosea	White Clover
Tropaeolum majus L.	Tropaeolaceae	Garden nasturtium
Urtica dioica L.	Urticaceae	Stinging nettle
Verbascum thapsus L.	Plantaginaceae	Great mullein
Veronica persica Poir.	Plantaginaceae	Persian speedwell
Viburnum opulus L.	Adoxaceae	Guelder-rose
Vinca major L.	Apocynaceae	Large periwinkle
Viola tricolor L.	Violaceae	Wild pansy
Wigelia florida A. DC.	Caprifoliaceae	Rose Weigela
Xanthium spinosum L.	Asteraceae	Prickly burweed
Yucca aloiflora L.	Asparagaceae	Dagger plant
Zantedeschia aethiopica (L.) Spreng.	Araceae	Arum lily
Zinnia elegans L.	Asteraceae	Common zinnia

areas of Ganderbal: The Shannon-Wiener Index was calculated using IVI values to assess species richness (Fig. 4). The diversity index for various urban areas in Ganderbal, with Duderhama exhibited the highest index at 1.53, followed by Gangerhama, Ganderbal, Saloora, Bamloora, and Wanipora. Beehama had the lowest index at 1.23. The diversity index for peri-urban areas, showed highest index inTulmulla (1.49), followed by Arch, Rangil and Sarich Chodri Bagh . Harran had the lowest index (1.32).

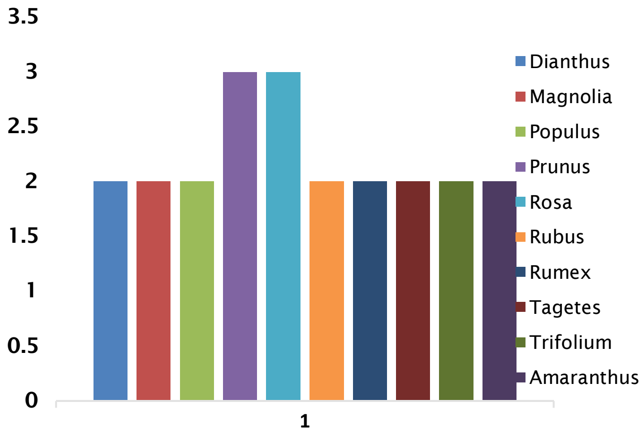

Fig. 1. Genera from the study area with 2 or greater than 2 species

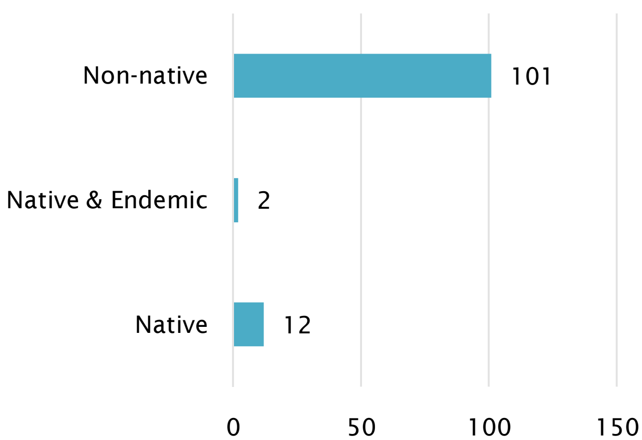

Fig. 2. Nativity and endemism of flora in the urban and peri-urban areas of Ganderbal

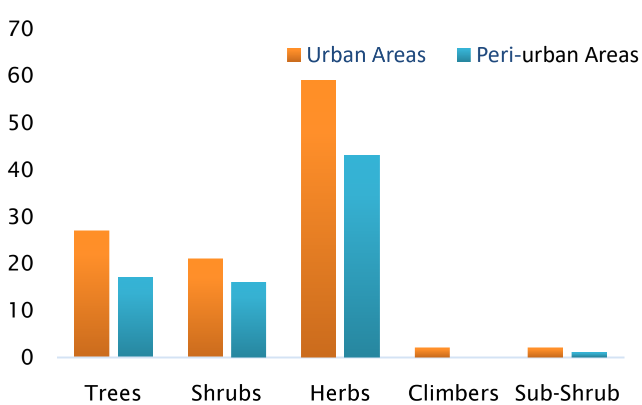

Fig. 3. Comparative analysis of different plant growth forms in the urban and peri-urban areas of Ganderbal

These values suggest moderate species diversity, with higher diversity observed in Duderhama (urban) and Tulmulla (peri-urban). The diversity levels align with those reported by Dar and Sundarapandian (2016) and Sharma et al. (2010) but are lower than reported by Malik and Bhat (2015) and Bhat et al. (2020) in other parts of the Indian Himalayas. The lower diversity observed in this study may be due to factors such as uneven species distribution, environmental stress, habitat fragmentation, invasive species or the ecological succession stage. Comprehensive sampling across various habitats contributed to capturing this range of diversity.

Biological spectrum of urban and peri-urban areas of Ganderbal

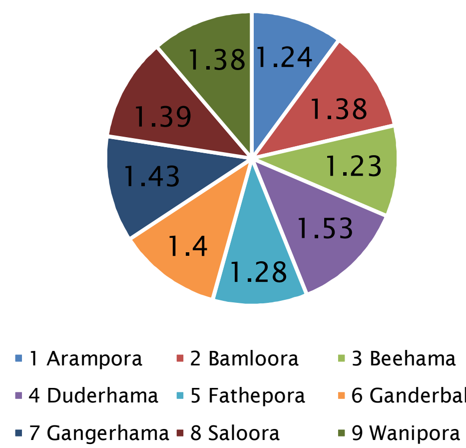

Fig. 4. Shannon diversity index under Urban areas of Ganderbal

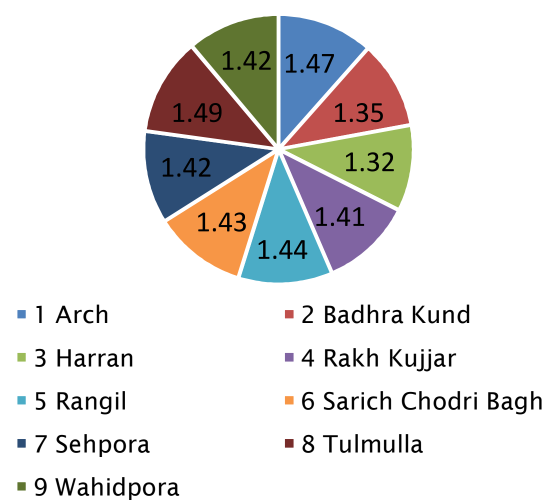

Fig. 5. Shannon diversity index value of under Peri-urban areas of Ganderbal

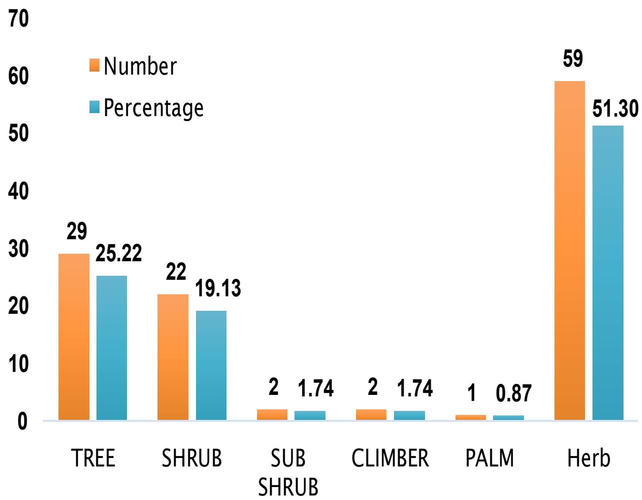

Fig. 7. Growth form distribution in urban and Peri-urban areas of Ganderbal

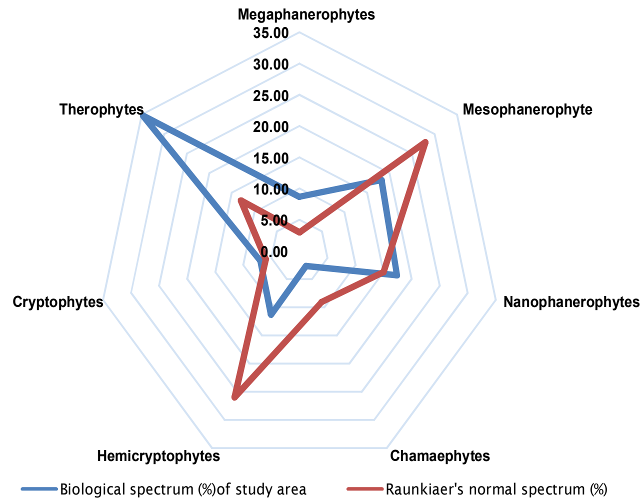

Fig. 8. Comparison of Biological spectrum with Raunkiaer's normal spectra

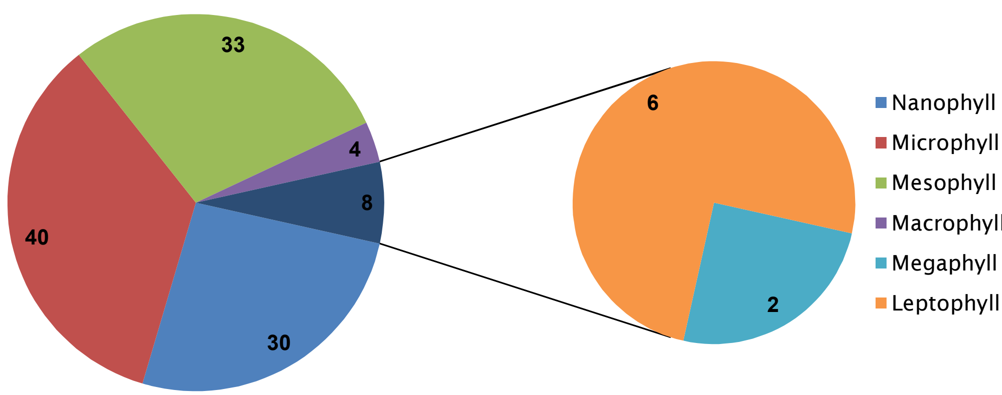

Fig. 9. Representation of leaf size in the Urban and Peri-urban areas of Ganderbal

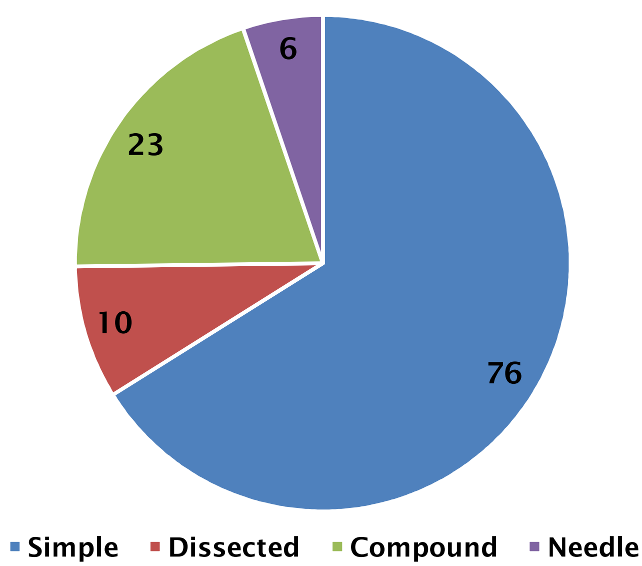

Fig. 10. Representation of leaf lamina types in the urban and Peri-urban areas of Ganderbal

The biological spectrum of urban and peri-urban areas of Ganderbal includes 25.22 % trees, 19.13 % shrubs, 1.74 % sub-shrubs, 1.74 % climbers, 0.87 % palms, and 51.30 % herbs (Fig. 7). Raunkiaer classification shows: maximum were therophytes (34.78 %) followed by nano-phanerophytes, hemicryptophytes, meso-phanerophytes, micro-phanerophytes and mega-phanerophytes, geophytes, and chamaephytes . The phytoclimate is thero-phanerophytic (Fig. 8). Leaf spectra revealed microphylls were maximum in number while megaphylls were least present (Fig. 9). Leaves with simple leaf lamina were maximum in number (Fig. 10).

The dominance of herbaceous plants aligns with previous research in the Indian Himalayan Region (IHR) by Rawat (2021) and Sharma et al. (2019).This prevalence of herbaceous species is likely due to the temperate climate of the Himalayan region, which favours herbs over woody plants (Mehraj et al., 2018). Herbs' short life cycle and adaptability to diverse and disturbed environments, such as urban areas, enable them to thrive, particularly in areas with high disturbance like roadsides. Khuroo et al. (2017) also observed that herbs constituted a significant portion of the flora, likely due to their ability to rapidly colonize and exploit available resources in such environments.

The high prevalence of therophytes is likely due to the frequent disturbances caused by anthropogenic activities, consistent with findings by Knapp et al. (2008). Therophytes thrive in disturbed environments, which may be attributed to the introduction of annual weeds and biotic influences, indicating environmental disruption (Al-Yemeni and Sher 2010). The region's microclimate, characterized by warm and dry summers, further supports the dominance of therophytes, which are adapted to survive arid conditions by synchronizing their life cycles with suitable seasons. Comparing our results with Raunkiaer's normal spectrum, we observed a significant increase in therophytes, suggesting that the phytoclimate of the area is thero-phanerophytic. This trend has been noted in previous studies in Jammu and Kashmir by by Singh and Kachroo (1994). The dominance of therophytes in warm and dry climates, coupled with human disturbances like overgrazing, highlights their ability to occupy niches created by such disruptions.

The dominance of small-leaved species is likely due to the region's arid climate and harsh winters. This pattern aligns with findings from other studies in the Indian Himalayan Region (IHR) by Khan et al. (2013) and Malik et al. (2007) with high prevalence of microphylls in similar climates. Most species in the study had simple leaf lamina. The shape and size of leaves are crucial for optimizing light capture and water management, especially in resource-deficient environments, as suggested by Malhado et al. (2009) and Manzoni et al. (2013). Ihsan et al. (2016) also observed a predominance of simple leaves in their studies.

The floristic diversity in urban and peri-urban areas patterns became apparent. Urban areas showed greater species diversity across all categories, with 27 tree species, 21 shrubs, 59 herbaceous plants, 2 sub-shrubs, and 2 climbers, compared to 17 tree species, 16 shrubs, 43 herbaceous plants, 1 sub-shrub, and no climbers in peri-urban areas. Moreover, the utilization of the Shannon-Wiener Index underscored variations in plant species diversity between urban and peri-urban environments in Ganderbal. The Shannon-Wiener Index further highlighted this difference, with the highest diversity index of 1.53 in Duderhama (urban) and 1.49 in Tulmulla (peri-urban). However, the highest similarity index (93%) was observed between Gangerhama (urban) and Sarich Chodri Bagh (peri-urban) in the tree and shrub categories, indicating some overlap. These variations provide insights into the differing ecological dynamics of urban and peri-urban environments in Ganderbal.

CONCLUSION

This study offers the first comprehensive assessment of floristic diversity and biological spectra in the urban and peri-urban areas of Ganderbal, Kashmir, revealing significant variations in species composition and ecological dynamics. A total of 115 plant species were identified, with urban areas showing greater diversity across all plant categories. Asteraceae and Rosaceae emerged as the most diverse families, while therophytes and microphylls were the dominant growth forms and leaf types. The study highlights the ecological significance of park habitats, which hosted the highest concentration of species, and underscores the need for targeted conservation strategies. Regular monitoring and a well-defined conservation policy are essential to ensure the effective management and preservation of prioritized communities and habitats in the region.

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Estimation of Tree Biomass and Carbon Density of Tropical Moist Deciduous Forest in Semi-Arid Regions-Katridaddi Hillock, Belagavi, Karnataka

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Abstract: This present work was carried out in semi-arid regions of Katridaddi hillock, Belagavi district Karnataka to study total biomass and carbon sequestration potential by quadrat method. A total of 30 quadrats of 32m × 32m were laid in the study area, and tree species were calculated accordingly. During the study, 22 tree species belonging to 12 families are recorded. Among the recorded tree species, the top dominant family with highest number of species comprises of Fabaceae with 88 species. The total carbon sequestered in the Hillock is 16.522 tons.

Keywords: Basal area, Biodiversity, Carbon sequestration, Total biomass, Quadrat method

Phytosociology is one of the important aspects to understand the composition, structure, distribution pattern, and vegetation dynamics of forest ecosystem (Rout, et al., 2018). Carbon sequestration in growing forests is known to be economically best option for reduction of global warming and global climatic change (Chavan and Rasal 2011). The aboveground biomass (AGB) of forest ecosystem is one of the primitive parameters describing its functioning. Studies on biomass of forest ecosystem are essential for determining storage of the carbon in tree component and carbon cycling at regional as well as global level (Behera et al., 2020). Forest play an important role in global carbon cycle and especially evergreen forests have a significant share in sequestering total earth's carbon as they account for half of global biomass (Prasad and Lakshmi 2015)

Kumar et al. (2015) estimated forest carbon stock for tree species of Balaganga Reserved Forest (BRF) in district Tehri Garhwal, Uttarakhand. Carbon sequestration of 610 trees belonging to 45 species was estimated. The average carbon content of these trees was 50.391t/tree. Jithila & Prasadan (2018) observed total carbon sequestered was 138.367t/year from Wayanad. Srinivas and Sundarpandian (2018) assessed the biomass and carbon stock in East Godavari region of Eastern Ghats in Andra Pradesh and showed that *Terminalia arjuna and Xylia xylocarpa* are the important tree species to endure and sink more carbon. Biradar and Mouna et al. (2021) have calculated Importance value index of 1387 species with Fabaceae with highest IVI.The objective of current studyto find out the physiology and carbon estimation by that hillock with tree diversity.

MATERIAL AND METHODS

Study area: The present study was undertaken in the semi-arid regions of Katridaddi Hillock, Belagavi district Karnataka. This Hillock is located in Katridaddi , small village in Bailhongal taluk in Belgavi district of Karnataka state, India. It is situated between 15.4924° north latitude and between 74.7765° east longitude and surrounded by Khanapur taluk towards west, Belagavi taluk towards west, Saundatti taluk towards East, Dharwad taluk towards South (Fig. 1). The preliminary phyto-sociological analysis of the study sites was performed by using quadrants. The size and number of quadrants needed for each site was determined separately using the species area curve method (Misra 1968)

Identification of specimen: The photographs of each plant species were taken during January 2024 to July 2024 by Phone (Poco X2) and specimen identification was done (Cooke 1906, Gamble 1928, Seetharam et al., 2000, Bhat 2003, Ramaswamy and Razi 1973, Saldanha 1984, 1996, Yoganarasimhan et al., 2018)

Tree height: Height of the trees is measured using a handmade hypsometer.

Girth classes: The tree girth was measured at breast height (GBH) approximately 1.37m above the ground. Trees only with diameter greater than 10cm were considered. GBH was measured and four girth classes (30-60, 60-90, 90-120) were considered to study the vegetation structure of the hillock.

Tree Bio volume: The volume of the tree stem can be calculated using the following formula

TBV=0.4x(Diameter)2 x height

Abovethe Ground Biomass (AGB): It was calculated by multiplying tree bio volume and wood density. The wood density of the tree were obtained from world agroforestry database. The standard average value of 0.6g/cm^3 is taken for the tree species whose wood density values are not available.

AGB=Tree Bio Volume×Wood density

Below the Ground Biomass (BGB): It includes all the living biomass below the soil excluding roots having diameter less than 2mm.

BGB=0.26 ×Above Ground Biomass

Total Biomass: It includes the complete biomass of the standing tree species. It is the sum of the above and below ground biomass.

Total Biomass = Above the Ground Biomass + Below the Ground Biomass

Carbon Storage: The 50% of the total biomass of a species is its carbon sequestration value.

Carbon storage=Biomass/2orBiomass×50%

Importance value index: In calculating this index, the percentage values of the relative frequency, relative density and relative dominance area summed up together (Curtis 1950).

Important Value Index= Relative Frequency+ Relative Density + Relative Dominance

$$\text{Relative frequency}=\frac{\text{Frequency of species A}}{\text{Total frequency of all species}}\times 100$$

$$\text{Relative density}=\frac{\text{Number of individuals of the species}}{\text{Number of individuals of all the quadrats}}\times 100$$

$$\text{Relative dominance}=\frac{\text{Total basal area of the species}}{\text{Total basal area of all the species}}\times 100$$

RESULTS AND DISCUSSION

A total of 22 tree species belonging to 12 families are recorded (Fig. 2, 3). Among these tree species, top 5 dominant families with highest number of species comprises of Fabaceae with 88 species, followed by Combretaceae (25 species), Rutaceae (18 species) and Anacardiaceae (16 species) . Biomass and carbon sequestration of a total 192 tree species in Katridaddi hilllock were calculated using the ground data with their height and girth at breast height measurements. The wood density values of each tree were obtained from World Agroforestry database for calculating

Table 1. Frequency, abundance and density of plant species in Katridaddi hillock, Belagavi district

Plant species	Frequency	Abundance	Density
Adina cordifolia (Roxb.) Brandis	16.66	1.2	0.2
Albizia odoratissima(L.f.) Benth	30	1.33	0.4
Bauhinia racemosa Lam.	26.66	1.12	0.3
Butea monosperma (Lam.) Kuntze	26.66	1.12	0.3
Careya arborea Roxb.	16.66	1.2	0.2
Cassia fistula L.	23.33	1.85	0.43
Chloroxylon swietenia DC.	36.66	1.63	0.6
Dalbergia lanceolaria L. f.	23.33	1.57	0.36
Dalbergia latifolia Roxb.	30	1.66	0.5
Diospyros melanoxylon Roxb.	13.33	1.25	0.16
Glycosmis cochinchinensis (Lour.) Pierre ex Engl.	13.33	2.0	0.26
Gymnosporia senegalensis (Lam.) Loes	16.66	1.2	0.2
Holarrhena pubescens Wall. ex G. Don	13.33	1.75	0.23
Lannea coromandelica (Houtt) Merr.	20	1.33	0.26
Phyllanthus emblica L.	10	1.33	0.13
Pteroearpus marsupium Roxb.	26.66	1.37	0.36
Sterculia *foetida* L.	10	0.37	0.1
Tectona grandis L.f.	20	1.33	0.26
Terminalia anogeissiana Gere & Boatwr.	33.33	1.2	0.4
Terminalia arjuna (Roxb.ex DC.) Wight & Arn.	16.66	1.2	0.2
Terminalia paniculata B.Heyne ex Roth	23.33	1.0	0.23
Xylia xylocarpa (Roxb.) W. Theob	36.66	1.81	0.66

tree bio volume, above ground biomass, below ground biomass, total biomass and total carbon sequestered by the trees. In Katridaddi Hillock total above and below ground biomass was26.45 tons 6.813 tons. The total biomass was 33.63 tons. The highest above ground biomass was 4.596 tons and lowest0.139 tons. The highest below ground biomass 1.194 tons and lowest below ground was 0.036 tons. *Albizia odoratissima* (L.f.) Benth. has highest tree bio volume of 7.535 cm^3 and lowest tree of 0.0.232 cm^3. *Xylia xylocarpa* (Roxb.) W. Theob.sequesters highest carbon of 2.895 tons followed by *Bauhinia racemosa* Lam.sequesters, *Gymnosporia senegalensis* (Lam.) Loes. sequesters,

Fig. 1. Katridaddi hillock Bailhongal Taluk, Belagavi, Karnataka

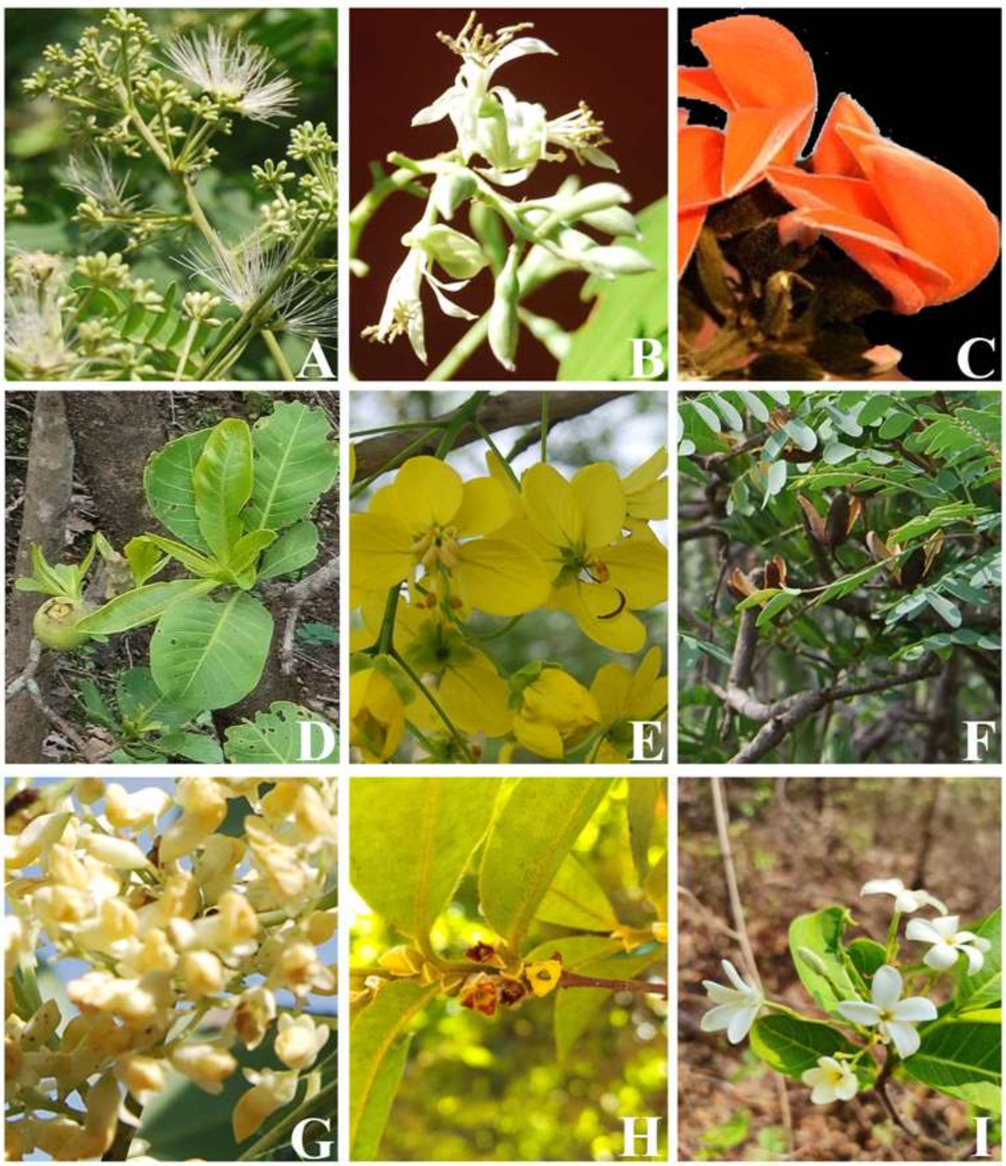

Fig. 2. A. *Albizia odoratissima* (L.f) Benth; **B.** *Bauhinia racemosa* Lam; **C.** *Butea monosperma* (lam) Kuntze; **D**. *Careya arobrea* Roxb.; **E**. *Cassia fistula* L.; **F**. *Chloroxylon swietenia* DC.; **G**. *Dalbergia latifolia* Roxb; **H.** *Diospyros melanoxylon* Roxb; **I.** *Holarrhena pubescens* Wall ex. G. Don.

Fig. 3. A. *Lannea coromandelica* (Houtt) Merr. ; **B.** *Phyllanthus emblica* L.; **C.** *Pterocarpus marsupium* Roxb.; **D.** *Sapindus trifoliatus* L. **E.** *Stericula foetida* L.; **F.** *Tectona grandis* L. f. ; **G.** *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.; **H.** *Terminalia paniculata* B. Heyne ex Roth. ; **I.** *Xylia Xylocarpa* (Roxb.) W. Theob.

Table 2. Importance value index of tree species in Katridaddi Hillock Belagavi district

Plant species	Mean GBH (in m)	Total basal area	Relative dominance	Relative frequency	Relative density	IVI
Adina cordifolia (Roxb.) Brandis	0.80	5.09	1.73	75.86	3.125	80.71
Albizia odoratissima (L.f.) Benth	1.16	10.70	3.65	136.6	6.25	146.5
Bauhinia racemosa Lam.	0.68	3.67	0.012	121.40	4.687	126.09
Butea monosperma (Lam.) Kuntze	0.41	1.33	0.45	121.4	4.687	126.5
Careya arborea Roxb.	0.23	0.42	0.14	75.86	3.125	79.125
Cassia fistula L.	0.37	1.08	0.37	106.2	6.77	123.34
Chloroxylon swietenia DC.	0.29	0.66	0.22	166.93	9.375	176.52
Dalbergia lanceolaria L.f.	0.70	3.89	1.32	106.2	5.729	113.24
Dalbergia latifolia Roxb.	0.59	2.76	0.009	136.61	7.812	144.43
Diospyros melanoxylon Roxb.	0.32	0.81	0.27	60.70	2.604	91.094
Glycosmis cochinchinensis (Lour.) Pierre ex Engl.	0.56	2.49	0.85	60.70	4.16	65.71
Gymnosporia senegalensis (Lam.) Loes	0.79	4.96	1.69	75.86	3.125	80.67
Holarrhena pubescens Wall.ex G. Don	0.37	1.08	0.37	60.70	3.64	64.71
Lannea coromandelica (Houtt) Merr.	0.84	5.61	1.915	91.07	4.166	97.1
Phyllanthus emblica L.	0.64	3.25	1.11	45.53	2.083	48.72
Pteroearpus marsupium Roxb.	0.41	1.33	0.45	121.40	5.72	127.57
Sterculia foetida L.	0.28	0.62	0.211	45.53	1.562	47.30
Tectona grandis L.f.	0.71	4.00	1.36	91.07	4.16	96.59
Terminalia anogeissiana Gere & Boatwr.	0.54	2.31	0.78	151.77	6.25	158.8
Terminalia arjuna (Roxb.ex DC.) Wight & Arn.	0.36	1.03	0.35	75.86	3.12	79.33
Terminalia paniculata B. Heyne ex Roth	0.76	4.59	1.56	106.2	3.64	111.42
Xylia xylocarpa (Roxb.) W. Theob	0.58	2.67	0.912	166.93	10.57	178.25

Table 3. Total carbon per species in Katridaddi Hillock, Belagavi district

Plant species	Wood density (in g/cm^3)	T_{BV} (in cm^3)	AGB (in tons)	BGB (in tons)	TB (in tons)	Total carbon per species (in tons)
Adina cordifolia (Roxb.) Brandis	0.600	2.048	1.228	0.319	1.547	0.773
Albizia odoratissima (L.f.) Benth	0.610	7.535	4.596	1.194	5.790	0.980
Bauhinia racemosa Lam.	0.782	3.144	2.458	0.639	3.094	1.547
Butea monosperma (Lam.) Kuntze	0.610	1.008	0.614	0.159	0.773	0.386
Careya arborea Roxb.	0.600	0.232	0.139	0.036	0.175	0.087
Cassia fistula L.	0.434	0.547	0.237	0.061	0.298	0.149
Chloroxylon swietenia DC.	0.486	0.504	0.244	0.063	0.307	0.153
Dalbergia lanceolaria L.f.	0.600	3.136	1.881	0.489	2.370	1.185
Dalbergia latifolia Roxb.	0.782	2.227	1.741	0.452	2.193	1.096
Diospyros melanoxylon Roxb.	0.829	0.409	0.339	0.088	0.427	0.213
Glycosmis cochinchinensis (Lour.) Pierre ex Engl.	0.508	0.878	0.446	0.115	0.561	0.280
Gymnosporia senegalensis (Lam.) Loes	0.600	3.994	2.396	0.622	3.018	1.509
Holarrhena pubescens Wall.ex G. Don	0.440	0.657	0.289	0.075	0.364	1.220
Lannea coromandelica (Houtt) Merr.	0.581	2.540	1.475	0.383	1.858	0.929
Phyllanthus emblica L.	0.610	1.966	1.199	0.311	1.510	1.155
Pteroearpus marsupium Roxb.	0.500	0.874	0.437	0.113	0.550	0.275
Sterculia foetida L.	0.727	0.564	0.410	0.106	0.516	0.258
Tectona grandis L.f.	0.600	3.226	1.935	0.503	2.438	0.219
Terminalia anogeissiana Gere & Boatwr.	0.580	1.516	0.879	0.228	1.107	0.553
Terminalia arjuna (Roxb.ex DC.) Wight & Arn.	0.512	0.933	0.477	0.124	0.601	0.300
Terminalia paniculata B. Heyne ex Roth.	0.610	2.079	1.268	0.329	1.597	0.798
Xylia xylocarpa (Roxb.) W. Theob	0.772	2.018	1.557	0.404	1.961	2.895

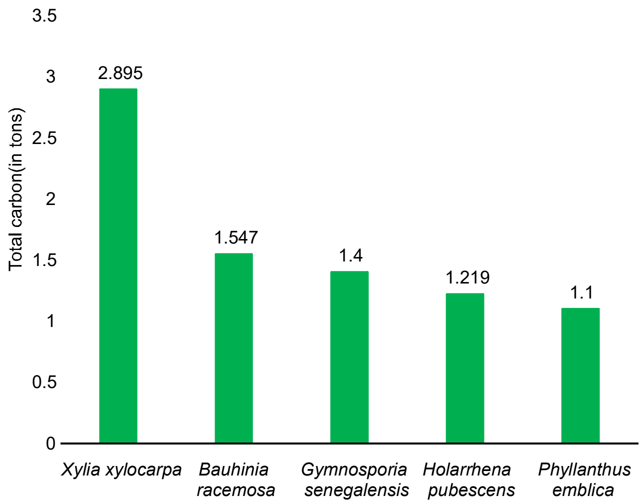

Fig. 4. Dominant families with highest number of species with total carbon estimation

Holarrhena pubescens Wall. ex G. Donsequesters, *Phyllanthus emblica L.* sequesters tree in Katridaddi Hillock (Table 1, 2, 3, Fig. 4).

CONCLUSIONS

The hillock area is considerably rich in diversity but also indicate human disturbances, thus altering their structure and distribution.. Fabaceae with 88 species being the dominant family reported during study. Highest tree bio volume in *Albizia odoratissima* (L.f.) Benth. (Fabaceae) need to be conserved. The woody plants has highest carbon sequestration potential than others as they store more carbon in their woody biomass. It is recommended that populations in plant species must be monitored regularly and updated in an open access database for threat assessment for interpretation of conservation status in the future.

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Phytosociological Assessment and Diversity of Herbaceous Vegetation in Gomarda Wildlife Sanctuary, Chhattisgarh, India

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***Abstract*:** Gomarda wildlife sanctuary is situated in the Sarangarh-Bilaigarh district of Chhattisgarh, India. The area is covered with tropical mixed deciduous vegetation. The present study was carried out in five sampling sites viz. Chantipali, Manjarmati, Bhatakona, Tendudhar and Gomarda to investigate the phytosociological parameters to determine the distribution and diversity of herb species in the sanctuary. The maximum IVI indicated the greater distribution of species like *Oplismenus burmanni* (Retz.) P. Beauv. in Chantipali and Manjarmati, *Heteropogon contortus*(L.) P.Beauv. *ex* Roem. &Schult. in Bhatakona and Gomarda, *Scleriatessellata* Willd. in Tendudhar and the minimum IVI showed the scarce distribution of herbal species like *Cajanus scarabaeoides*(L.) Thouars*, Hemidesmus indicus* (L.) R.Br. and *Stylosantheshamata* (L.) Taub. in Chantipali, *Hemionitis tenuifolia* (Burm.*f.*) Christenh. in Manjarmati and Bhatakona, *Cissampelos pareira* L. in Tendudhar and Gomarda. The maximum value of herbal diversity (Shannon-Wiener index) was (3.36) in Manjarmati and minimum (1.31) in Chantipali. The study revealed the presence of 66 herb species belonging to 52 genera and 25 families.

***Keywords*:** Chhattisgarh, Diversity, Gomarda Wildlife sanctuary, Herbs, Phytosociology

Herbs are playing an important role in forest ecosystem and herbaceous floor vegetation show high nutrient content and rapid turnover rates as influenced by climatic conditions and vegetation characteristics (Chen et al., 2021). Herbs have interrelationship with plants growing in their vicinity. Assessing understorey vegetation is crucial for understanding ecological processes and interactions in forest ecosystems (Jhariya et al., 2013, Jhariya and Singh 2021). Herbaceous vegetation makes up ninety percent of plant species and up to twenty percent of foliar litter, but making up less than one percent of the forest's biomass. The herb layer plays a crucial role in forest formation despite fierce competition (Borah et al., 2021). The occurrence of species and their natural relationship is an important aspect of the community structure. Studies in sub-tropical regions have highlighted an entirely distinct pattern for vegetation distribution distinguished by altitude fluctuation (Almeida et al., 2020). Altitude and topography affect soil texture, physical and chemical qualities, influencing plant composition (Kermavnar and Kutnar 2020). Diversity of any region is a crucial ecological characteristic which has a strong correlation with existing environmental and anthropogenic influences. It not only indicates productivity but also represents the health of the vegetation. The loss and degradation of habitats reduce community diversity. However, field surveys provides an adequate information on vegetation structure (Ullah et al., 2024). This study aims to document the phytosociological characteristics of herbaceous vegetation in Gomarda Wildlife Sanctuary through site-specific sampling.

MATERIAL AND METHODS

Study site: Gomarda wildlife sanctuary lies between 21°30'24"N. latitude and 83°06'4"E. longitude. It covers an area of about 277.82 km^2 of forests in both Baramkela and Sarangarh forest ranges (Fig. 1). The land is gently undulating, with numerous rocks and boulders, incapable of cultivation, and hence naturally protected. In the summer season, the temperature varies from 29.5°C to 49 °C, while in the winter season, it drops 8°C to 25°C. The perennial Lath river flowing through the centre of the sanctuary is the main source of water. The vegetation is tropical mixed deciduous forest with sal (*Shorea robusta*), teak (*Tectona grandis*), bamboo (*Dendrocala musstrictus*), *terminalia* spp. and other species (FSI 2024).

Field sampling: The study was conducted during July 2022 to February 2023 at five different sites viz. Chantipali, Manjarmati, Bhatakona, Tendudhar and Gomarda within the core, buffer and transition zones. All the five sites were selected on the basis of different topology viz. slope, fold aspect, soil texture and soil structure for the study (Table 1). The sampling was carried out in all three seasons; rainy, winter and summer (Margalef, 1958). Circular quadrats of 354.4 cm circumference were laid at each study site randomly until three times new species stop being found (Odum 1971, Muller-Domboisand Ellenberg 1974) (Fig. 2).

Identification of collected samples: Identification and nomenclature of collected herbaceous plants was done by following The Flora of British India Vol. I-VII (Hooker, 1875-97); Flora of Madhya Pradesh Vol. I (Verma et al., 1993); Vol. II (Mudgal et al., 1997); Vol. III (Singh et al. 2001); Supplement to The Flora of Madhya Pradesh (Khanna et al., 2001); Floral Diversity of Chhattisgarh (Mishra and Naik, 2021); Biodiversity of Chhattisgarh: A Check List (Pandey et al., 2023) and Plants of the World Online (https://powo.science.kew.org) respectively. Plants that could not be assigned a species rank were considered morphospecies, which are morphologically identical and treated as a single species in later analyses (Linares-Palomino and Ponce-Alvarez 2009).

Data analysis: frequency, density and abundance were estimated (Mishra 1968) and used to compute relative density (RD) relative frequency (RF) and relative abundance (RA). The importance value index (IVI) for the herb species was determined as the sum of the relative frequency, relative density and relative abundance (Cottam and Curtis 1956, Mishra et al., 2012).

Relative frequency (RF) = (Frequency of a species /Total frequency of all the species) x 100.

Relative density (RD) = (Density of a species/Total density of all the species) x 100.

Relative abundance (RA) = (Abundance of a species/Total abundance of all the species) x 100.

Importance value index (ivi) = RF + RD + RA.

Species diversity: This was calculated for each forest type.

Shannon Weaver's diversity index- H' (Shannon and Weiner 1963).

$H' = -\Sigma [(ni/N) \ln (ni/N)]$

Simpson's index of dominance - D (Simpson 1949).

$D = \Sigma (ni/N)$,

Where, ni = Total number of individuals of species i

N = Total number of individuals of all species in that vegetation type.

Simpson's diversity index = 1- D (Simpson 1949).

RESULTS AND DISCUSSION

A total of 66 herb species were recorded from all the five sites belonging to 52 genera and 25 families. Poaceae was represented highest number of species (19), followed by Cyperaceae 7 species, Malvaceae 5 species, Asteraceae and Rubiaceae both 3 species, Convolvulaceae, Acanthaceae, Phyllanthaceae and Polygalaceae 2 species each, and remaining 16 families were represented by one species each. The maximum number of herb species (39) were recorded at the site Tendudhar followed by Manjarmati (33), Gomarda (32), Bhatakona (28) and minimum at Chantipali (24). The Importance value index (IVI) was highest at Chantipali, for *Oplismenus burmanni* (Retz.) P.Beauv. as 31.10 followed by *Heteropogon contortus* (L.) P.Beauv. *ex* Roem & Schult; while *Cajanus scarabaeoides* (L.) Thouars, *Hemidesmus indicus* (L.) R.Br. and *Setaria flavida* (Retz.) Veldkamp had showed the least IVI. At Manjarmati, the IVI was recorded highest for *Oplismenus burmanni* (Retz.) P.Beauv. as 23.64 followed by *Grona triflora* (L.) H.Ohashi & K.Ohashi as 22.51; while least IVI was recorded for

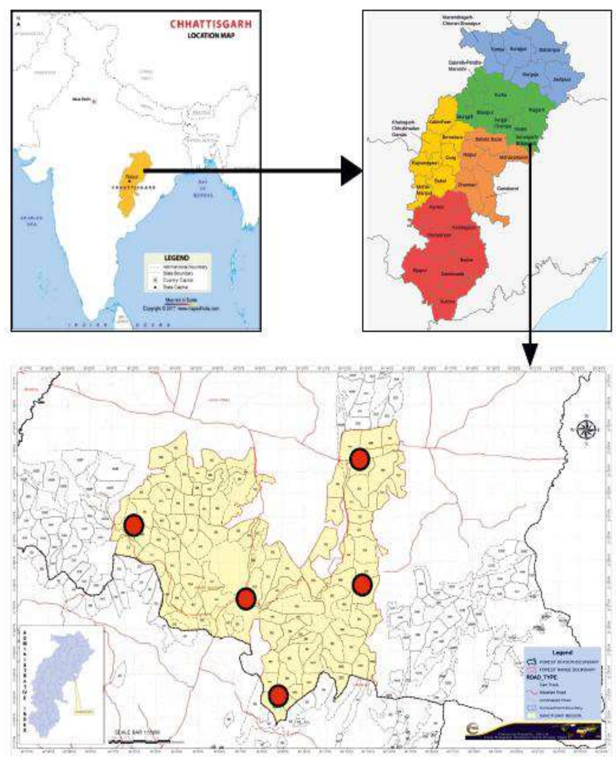

Fig. 1. Map showing location of Gomarda Wildlife Sanctuary and five sampling sites

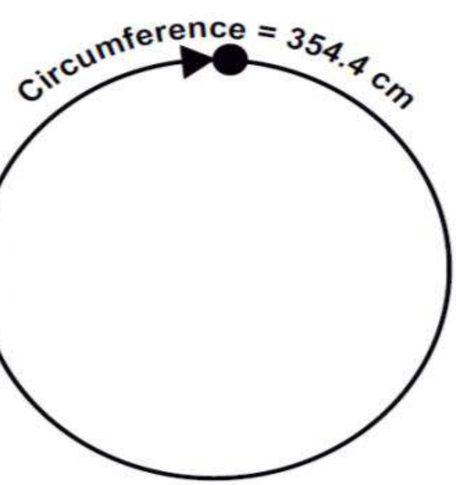

Fig. 2. Circular quadrate

Table 1. Phytosociological study of herbaceous layer in five different study sites of Gomarda Wildlife Sanctuary

Botanical name of the herbs	IVI				
	Chantipali	Manjarmati	Bhatakona	Tendudhar	Gomarda
Alloteropsis cimicina (L.) Stapf	4.44	13.95	15.41	6.96	6.76
Aristida adscensionis L.	14.74	7.70	12.63	11.22	8.09
Ayenia herbacea (Roxb.) ined.	-	-	6.42	-	3.76
Bonnaya ciliate (Colsm.) Spreng.	-	8.11	11.17	-	-
Bothriochloa pertusa (L.) A.Camus	10.64	-	-	-	-
Brachiaria ramosa (L.) Stapf	12.39	7.49	-	-	-
Cajanus scarabaeoides (L.) Thouars	2.95	-	-	3.83	6.84
Cenchrus americanus (L.) Morrone	14.23	12.13	-	-	-
Chrysopogon zizanioides (L.) Roberty	-	5.09	-	2.35	-
Cissampelos pareira L.	-	3.70	-	1.48	2.38
Cissus repanda (Wight &Arn.) Vahl	-	3.80	4.51	-	-
Crotalaria prostrata Rottler *ex* Willd.	-	-	5.45	-	3.74
Curculigo orchioides Gaertn.	-	4.74	-	5.56	-
Cyanthillium cinereum (L.) H.Rob.	-	7.10	-	-	-
Cynodon arcuatus J. Presl	18.01	13.55	18.69	14.27	
Cynodon radiatus Roth	-	-	-	-	21.70
Cyperus albescens (Steud.) Larridon & Govaerts	-	8.47	-	-	-
Cyperus alopecuroides Rottb.	-	10.56	8.49	-	-
Cyperus iria L.	-	9.00	-	11.07	12.80
Digitaria abludens (Roem. & Schult.) Veldkamp	15.99	8.11	19.32	-	14.91
Digitaria longiflora (Retz.) Pers.	11.29	-	-	-	4.53
Dioscorea bulbifera L.	-	4.73	2.98	-	5.74
Echinochloa colonum (L.) Link	15.34	12.15	-	8.05	-
Elephantopus scaber L.	9.03	8.56	-	10.29	8.15
Emilia sonchifolia (L.) DC.	10.54	-	-	-	-
Eragrostiella brachyphylla (Stapf) Bor	14.32	-	-	9.91	15.43
Eragrostiella nardoides (Trin.) Bor	-	-	-	4.54	7.60
Euphorbia hirta L.	-	5.35	9.44	-	-
Evolvulus alsinoides (L.) L.	7.51	-	-	5.25	6.53
Evolvulus nummularius (L.) L.	-	14.48	19.86	8.29	-
Fimbristylis alboviridis C.B.Clarke	-	-	8.69	-	10.70
Fimbristylis dichotoma (L.) Vahl	-	9.95	11.17	10.29	-
Grewia hirsuta Vahl	4.86	-	-	2.80	3.76
Grewia rothii DC.	-	-	2.98	1.48	-
Grona triflora (L.) H. Ohashi & K. Ohashi	15.15	22.51	15.63	11.73	19.06
Habenaria furcifera Lindl	-	-	-	5.92	-
Hemidesmus indicus (L.) R.Br.	2.95	-	5.45	3.84	-
Hemionitis tenuifolia (Burm.*f.*) Christenh.	-	2.48	3.66	-	-
Heteropogon contortus(L.) P.Beauv. *ex* Roem. & Schult.	24.52	17.69	31.58	16.13	23.29
Justicia glauca Rottler	-	3.39	5.56	4.54	-

Cont...

Table 1. Phytosociological study of herbaceous layer in five different study sites of Gomarda Wildlife Sanctuary

Botanical name of the herbs	IVI				
	Chantipali	Manjarmati	Bhatakona	Tendudhar	Gomarda
Mesosphaerum suaveolens (L.) Kuntze	-	-	9.99	-	11.80
Murdannia nudiflora (L.) Brenan	-	-	-	6.70	9.99
Oplismenus burmanni (Retz.) P.Beauv.	31.10	23.64	-	13.20	-
Phyllanthus niruri L.	-	6.83	13.95	-	-
Phyllanthus virgatus G.Forst.	-	-	7.66	7.12	-
Pigea enneasperma (L.) P.I.Forst.	9.77	-	7.31	-	12.17
Polygala arvensis Willd.	-	7.61	-	-	9.32
Polygala erioptera DC.	-	-	-	6.49	-
Rostellularia mollissima (Nees) Nees	-	-	-	-	3.76
Scleria terrestris (L.) Fassett	19.64	-	-	6.96	3.74
Scleria tessellata Willd.	-	-	-	24.24	-
Scleromitrion verticillatum (L.) R. J. Wang	-	7.10	-	5.22	-
Senna cobanensis (Britton) H. S. Irwin & Barneby	-	-	-	3.84	-
Senna tora (L.) Roxb.	5.78	-	-	-	4.53
Setaria flavida (Retz.) Veldkamp	21.90	10.87	14.85	9.73	5.40
Setaria pumila (Poir.) Roem. & Schult.	-	-	-	14.55	-
Sida acuta Burm.*f.*	-	5.44	11.95	-	-
Spermacoce hispida L.	-	6.83	11.20	5.88	13.78
Spermacoce pusilla Wall.	-	-	-	-	13.01
Sporobolus diandrus (Retz.) P.Beauv.	-	-	-	3.46	4.56
Striga densiflora (Benth.) Benth.	-	-	-	5.39	-
Stylosanthes hamata (L.) Taub.	2.95	-	4.00	9.05	-
Theriophonum minutum (Willd.) Baill.		-	-	5.22	4.53
Trichosanthes cucumerina L.	-	-	-	3.06	-
Triumfetta rhomboidea Jacq.	-	6.88	-	-	-
Urochloa ramosa (L.) T. Q. Nguyen	-	-	-	10.08	17.66

Hemionitis tenuifolia (Burm.*f.*) Christenh. as 2.48., The IVI at Bhatakona was highest for *Heteropogon contortus* (L.) P.Beauv. *ex* Roem. & Schult. as 31.58 followed by *Evolvulus nummularius* (L.) L. as 19.86; while *Hemionitis tenuifolia* (Burm.*f.*) Christenh. showed least IVI value as 3.66. At Tendudhar, the IVI was highest for *Scleria tessellata* Willd. as 24.24 followed by *Heteropogon contortus* (L.) P.Beauv. *ex* Roem. & Schult. while minimum was for *Cissampelos pareira* L. (1.48) at Gomarda. The highest IVI e was recorded for *Heteropogon contortus* (L.) P. Beauv. *Ex* Roem. & Schult)(23.29) followed by *Cynodon radiatus* Roth as while *Cissampelos pareira* L. showed the least IVI as 2.38 (Table 2).

The maximum diversity of herbaceous layer (Shannon Weaver's diversity index- H') was 3.36 for the site Manjarmati and minimum was 1.31 for Chantipali (Fig. 3). In different habitats of Pench Tiger Reserve, Madhya Pradesh and Similipal Tiger Reserve, Odisha, the H' ranged from 1.78 to 3.05 and 1.78 to 3.12 respectively ,reflecting slightly moderate diversity as compared to this study (Singh et al., 2014, Chourasia et al., 2016). The highest value of dominance (Simpson's index of dominance - D) was 0.055 for site Chantipali and lowest value 0.035 for Tendudhar (Fig. 3). Similar trend was observed in various communities and regions of Mudumalai Wildlife Sanctuary, Tamil Nadu and Periyar Tiger Reserve, Kerala (Sundarapandian and Swamy 2010, Murali et al., 2013) The maximum diversity of herbs (Simpson's index of diversity- 1- D) was 0.965 for site Tendudhar and minimum 0.945 for Chantipali (Fig. 3). These value indicated the structure of the herbaceous layer from greater to moderate distribution in the study area. The recorded information on

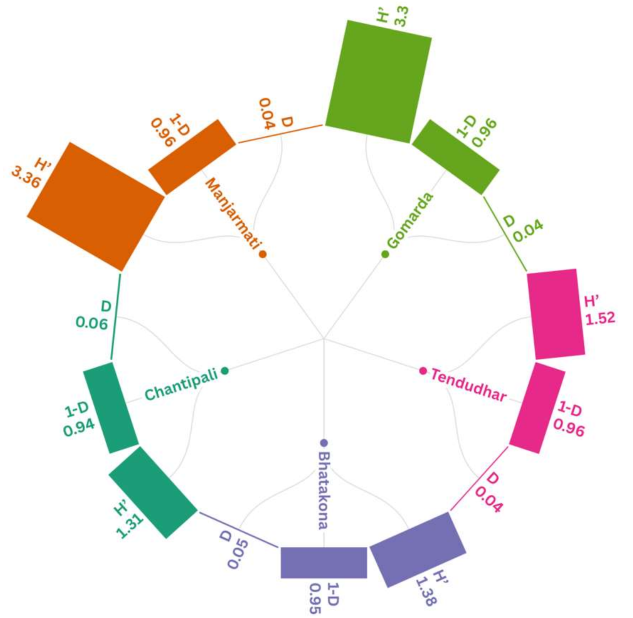

Fig. 3. Different diversity indices of herbaceous layer in five different study sites of Gomarda wildlife sanctuary

the diversity of the communities provides a better insight into the status of the forests in the Gomarda Wildlife Sanctuary.

CONCLUSION

Recurrent forest fires and illegal resource extraction threatens Gomarda wildlife sanctuary's natural cycle and biodiversity. Herbs play an important role in forest structure and diversity, demonstrating the sanctuary's ecological value. Strict fire control methods, enforcement against exploitation, and long-term livelihood prospects for people are vital. Conservation efforts should prioritize research collaborations, biodiversity assessments, and awareness campaigns. Long-term protection necessitates an Integrated Forest Management Plan that includes conservation funds and legislative support to preserve the sanctuary's ecological survival.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this article. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Do the Geo-Climatic Variables shape Morphological Plasticity Pattern in *Melia dubia* Cav. Natural Populations in Gujarat?

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Abstract: This work presents the morphological variations in foliar and fruit traits among four natural populations of *Melia dubia* in Gujarat, India. Results showed considerable but significant variations exist among and within populations for seven leaf and four fruit traits. *Sagai (SG)* population had maximum values for most foliar traits, whereas the *Kaparada (KP)* and *Nanapondha (NP)* populations had higher dimensions of fruit traits. The intraclass correlation exhibited a strong positive relationship between leaf length, petiole length, and leaf base diameter. Similarly, pulp weight had strong positive correlations with fruit dimensions (length, width, and weight). Multivariate analysis of morphological data reduced it into three principal components (PCs), capturing a cumulative variability of 80.7%. Correlation and regression analysis of PC scores with geo-climatic variables revealed a strong direct relationship between habitat latitude, altitude, and mean annual temperature. The mean annual rainfall also had a strong but inverse influence, whereas longitude did not record any significant association with PC1. The populations occupying niches of higher altitude, higher MAT, and lower precipitation were characterised by longer and narrower leaves with a maximum number of pinnae and leaflets and smaller fruits.

Keywords: Geoclimate, Correlation, Morphological Traits, *Melia dubia*

Morphological characteristics of any organism are thought to exist and develop as a result of inherited genetic information, and it is also an accepted fact that changing environmental and geographical conditions play an important role. In fact, geographic variation leads to variability in the environmental conditions that any species experiences. The stress or stimulation exerted by the environment motivates the plants to adapt. *Cacti*, for example, adjust their leaf morphology to cope with a resource-poor and harsh environment. In wild tree species, many common garden experiments have apparently established the influence of geo-climatic variables of origin in forming morphological variations (Warren et al 2005, Vitasse et al., 2009, Akalushi et al., 2018). The morphological variability in foliar and fruit traits of *M. dubia* were invested at the population level and attempted to understand how the geo-climatic gradients play a role in shaping these traits.

Malabar neem (*Melia dubia* Cav.) is an indigenous species of moist localities and tropical forests that grows naturally in most parts of India. Due to its fast-growing nature, broader industrial uses, and thus economic prospects, this species has caught the interest of researchers in recent years for domestication and productivity improvement (Thakur et al., 2017, Parmar et al., 2019, Chauhan et al., 2021, Parmar et al., 2019).

M. dubia is a large deciduous tree with bark that is dark brown in colour and peels in flimsy, restricted strips with expansive, shallow, longitudinal breaks. The leaves are typically bi- or tri-pinnate and range in length from 30 to 90 cm. Pinnae: 3-7 pairs, 10-20 cm long; rachis: 10–30 m long. The leaflets are ovate or oblong-lanceolate, 4 to 8 cm long, and come in 2 to 11 pairs. The violet and white flowers, which are about 8 mm long and fragrant, are borne on the upper axils of the leaves. When young, the fruit is drupaceous, ellipsoid, 1.5 cm long, smooth and shiny, and yellowish when fully ripe (https://indiabiodiversity.org/species/show/31551).

MATERIAL AND METHODS

Population details and habitat characteristics: For this study, chose four natural populations of *M. dubia* from the hilly regions of South Gujarat (Chauhan et al., 2018). The Kaprada (KP) and Nanapondha (NP) populations belong to the Valsad district, while Waghai (WG) from the Dangs and Sagai (SG) from the Narmada district. The population's geographic situations (latitude, longitude and altitude) are depicted in the Figure 1. The climate of population sites is typically dry sub humid, characterised by a fairly hot summer, a moderately cold winter, and a humid and warm monsoon. Most of the precipitation is received from the south west monsoon, concentrated during the months of July and August. Edapho-climatic details of the sites were taken from World Weather Online (https://www.worldweatheronline.com), Gujarat State Disaster Management Authority (https://www.gsdma.org), and The Commission of

Agriculture (http://www.gujenvis.nic.in) and given in Table 1.

Morphological variability assessment: This investigation includes ten individuals from each population who had a good phenotype and were at least 100 metres apart. Fresh leaves were examined in the field for morphological variations. With the help of a tree pruner, six small branches were carefully removed from each tree, covering all directions (E-W-N-S). One fully developed and intact leaf was chosen from each branch for measurement. Figure 2 shows a diagram illustrating leaf measurements, and the methodology adopted is provided in Table 2. Assessment of variation in fruit traits was carried out in the Seed Technology Laboratory, College of Forestry, NAU, Navsari. A sufficient quantity of mature fruits was collected from each individual in the population while maintaining proper identity. For evaluation of morphometric variations, 60 uniform drupes per individual were used in a population as per standard procedure (Table 2).

Statistical analysis: Shapiro-Wilk's Test was used to check for normality in the morphological data of leaf and fruit traits. Analysis of variance and multiple comparison tests were done to analyse the differences among the populations. Morphological differences within the population were also presented using descriptive statistics. Simple correlation (Pearson) analysis was done in order to check the intra- and inter-class relations between foliar and fruit traits. After transforming the data (Box-Cox), Principal Component Analysis (PCA) was done, which reduced the morphological dataset to 3 meaningful principal components (PCs). The decision for the inclusion of PCs for interpretation was taken on the basis of the scree plot (eigenvalues > 1). Further

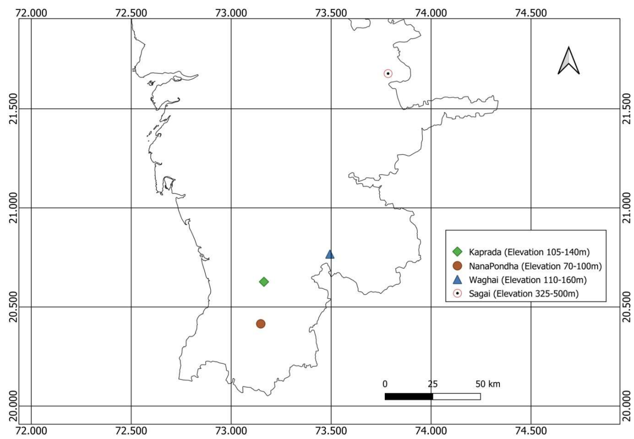

Fig. 1. Geographic positions of the selected natural population of *M. dubia* in Gujarat, India

Table 1. Edapho-climatic details of selected *M. dubia* populations in Gujarat, India

Population	Abbreviation	Soil type	Max. Temp. (^{o}C)	Min. Temp. (^{o}C)	M.A. Temp. (^{o}C)	M.A. rainfall (cm)
Kaprada	KP	Lateritic medium black	32.3	24.2	28.2	274.4
Nanapondha	NP		34.5	22.6	28.6	244.1
Waghai	WG	Lateritic deep black clayey and sandy soil	33.4	21.3	27.3	241.2
Sagai	SG	Black to loamy soil	35.3	25.8	30.6	113.3

Table 2. Details of the morphological traits characterized in natural populations of *M. dubia*

Morphological traits	Abbreviation	Unit	Procedure
Leaf length	LL	cm	Measured from the base of the petiole to the apex with the help of a ruler scale (stainless steel)
Leaf width	LW	cm	Measured at its widest part, with the help of a ruler scale
Petiole length	PL	cm	Using a ruler scale, measure from the base to the point of attachment of the first pair of pinnae.
Rachis length	RL	cm	Measured from the point of attachment of first pair of pinnae to the leaf apex, with the help of ruler scale
Base diameter	BD	mm	Measured with the help of a digital calliper
Number of pinnae (pair) per leaf	NP/L	Nos.	Counted in the half side of the leaf and expressed as pair.
Number of leaflets per pinnae	NL/P	Nos.	Counted in each pair of pinnae and took averages.
Fruit length	FL	mm	Measured between the end of the vertical axis with the help of a digital calliper,
Fruit width	FW	mm	Measured at the site of the maximum circumference with the help of a digital calliper
Fruit weight	FWt	g	Weighed Individual fresh fruits on an electronic weighing balance
Pulp weight	PWt	g	Individual fruit was de-pulped and weighed using an electronic weighing balance.

choose the first principal component scores from the correlation matrix for correlation and regression analysis with geo-climatic variables. Statistical analysis was performed with the online resources OPSTAT (Sheoran et al., 1998) and PAST (Hammer et al., 2001).

RESULTS AND DISCUSSION

Morphological variations in leaf and fruit traits: The mean values recorded for these characters varied significantly among the four natural populations of *M. dubia* (Table 3).

Leaf variables like leaf length (102.77 cm), petiole length (23.09 cm), rachis length (64.73), and base diameter (9.24 mm) were recorded as highest in the *SG* population. Although these traits did not differ significantly between the other populations, lower values were in the *KP* or *WG* populations (Table 3). In contrast, significantly wider leaves were attributes of the *NP, KP,* and *WG* populations, whereas the narrowest leaf (54.74 cm) was observed in the *SG* population. Additionally, the *SG* population had the maximum pair of pinnae (6.93) as well as the highest number of leaflets per pinnae (9.54).

Leaf morphology is believed to vary among different genotypes of the same species due to genomic differences or environmental changes in the habitat (Bussotti and Pollastrini 2015). In the present investigation, all the leaf traits recorded significantly higher in the *SG* population, except leaf width, which was significantly narrower. It is a well-established fact that smaller leaves are adaptations to higher elevations and high temperatures and the opposite is true for adjustments in humid and cold areas (Cordell et al., 1998). Similarly, large leaf area is a prerequisite in fast-growing species, and narrow leaves with large petiole sizes are adaptations in resource poor habitats, particularly in drier environments (Pyakurel and Wang 2013). Because the *SG* population is a fast-growing species that lives in relatively hot, drier conditions at high elevation (Table 1, Fig. 1), trees with the longest leaf length and narrowest width may have indirectly reduced leaf area in order to withstand transpiration loss. The greatest number of pinnae and leaflets in the *SG* population, on the other hand, are attributed to the system's pressure to manage the high pull of food resources in order to sustain its rapid growth. Longer petiole length, longer rachis length, and a thicker base diameter might have reinforced the longer leaf, as evident with their intra-class correlations (Table 4). Variability in foliar morphology due to varying environmental conditions is well documented in other species as well. Atchaya et al. (2019) recorded a significant influence of temperature on changes in the number of leaves, number of leaflets, and leaf area in *M. dubia*. Similarly, Ramchandran and Vasudeva (2020) opined that variation in the leaf morphology of *Pyranacantha volubilis* was due to environmental variables at different geographic origins of the seeds. There was significant variation in fruit traits among the four natural populations of *M. dubia* (Table 3). In variance analysis, the KP population had the same maximum fruit length (28.34 mm) and width (23.20 mm) as the *NP* population (28.31 mm and 23.16 mm, respectively). Heaviest fruits (9.10 g/fruit) were measured in *NP,* not significantly different from the *KP* population (8.39 g/fruit), whereas, lightest fruits were observed in the *SG* and *WG* populations. The maximum pulp weight (7.26 g/fruit) in the *NP* population was significantly *at par* to the *KP* population (6.83 g/fruit) and the minimum 5.70 g/fruit in the SG population did not significantly differ from the WG population.

In the present study, fruit traits differ significantly among the populations. The *KP* and *NP* populations were found to be equally superior for all of the investigated fruit traits, whereas the SG population had the lowest trait values. Fruit morphology similarities and differences may be due to genotype or ecological similarity and dissimilarity between populations (Table 1, Fig. 1). A range of coefficients of variability for fruit characters (CV = 5.13-14.80) indicated that the genotypic contribution to variability was limited.

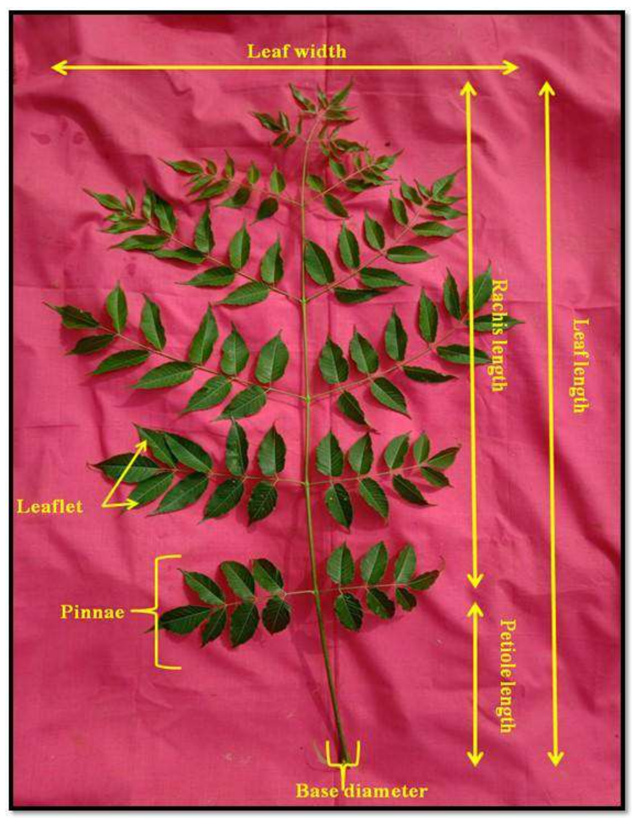

Fig. 2. Illustration of measurements of leaf morphology in *M. dubia*

Differences in biotic stress levels experienced by the mother tree during the fruit development stage, on the other hand, could have also resulted in such variations. Moisture availability and mineral nutrition influence fruit morphology (Gutterman 2000). Variability in fruit morphology due to differences in moisture and nutrition is well documented in other woody perennials like *Melia azedarach* (Irmayanti et al 2015), *Tamarindus indica* (Okello et al., 2018) and *Terminalia chebula* (Sharma et al 2016).

Inter and Intra correlations among leaf & fruit traits: There were significant positive intra-class correlations observed for most leaf traits (Table 4). Leaf length exhibited a very strong positive correlation (r=0.838) with petiole length. Base diameter was a strong positive correlation with the number of pinnae (r=0.768), leaf length (r=0.669) and petiole length (r=0.635). The number of pinnae was positively and significantly correlated with the number of leaflets or pinnae (r=0.739). In contrast, except for the number of pinnae, which was found to be negatively correlated in moderate magnitude (r = -0.425), leaf width showed either insignificant or a weak correlation with other interclass morphological characters. Inter-character correlations are of major interest in tree improvement programmes as the improvement of a character may lead to synchronised changes in the linked character. Interpretation of the correlations among leaf traits revealed the crucial role of the petiole, rachis length, and base diameter in determining the leaf length and strength. Similarly, a greater number of pinnae could have increased the number of leaflets in a leaf. The possible explanation for the direct positive relationship of these traits with leaf size might be the provisioning of structural reinforcement for better interception of light resources. Similar conclusions were drawn by Eichelmann et al. (2004) in *Betula pendula*; in addition, positive relations between leaf length and petiole length and negative leaf width were in *Fagus orientalis* by Bayramzadeh et al. (2012).

Table 3. Descriptive statistics and analysis of variance for 11 morphological traits within and among the population of *M. dubia* in Gujarat

Character		Descriptive statistics				Analysis of variance	
		Populations				Mean	C.V. %
		Kaprada	Nanapondha	Waghai	Sagai		
Leaf length (cm)	Mean	94.56^{b}	97.17^{b}	94.64^{b}	102.77^{a}	97.29	5.27
	C.V.	7.07	5.37	5.49	2.39		
Leaf width (cm)	Mean	65.58^{a}	63.10^{a}	62.66^{a}	54.74^{b}	61.52	9.61
	C.V.	9.70	9.22	9.42	10.1		
Petiole length (cm)	Mean	19.69^{b}	20.69^{b}	19.47^{b}	23.09^{a}	20.74	9.64
	C.V.	14.89	6.67	10.37	5.15		
Rachis length (cm)	Mean	56.33^{b}	58.42^{b}	58.36^{b}	64.73^{a}	59.46	9.71
	C.V.	6.71	13.84	10.42	6.36		
Base diameter(mm)	Mean	7.21^{b}	7.33^{b}	7.03^{b}	9.24^{a}	7.7	7.86
	C.V.	7.35	8.87	5.41	8.55		
Number of leaflets/pinnae	Mean	8.11^{c}	8.57^{b}	8.68^{b}	9.54^{a}	5.76	4.38
	C.V.	5.53	4.74	3.42	6.28		
Number of pinnae (pair)	Mean	5.48^{b}	5.07^{c}	5.57^{b}	6.93^{a}	8.73	5.17
	C.V.	3.64	0.29	5.50	2.82		
Fruit length (mm)	Mean	28.34^{a}	28.31^{ab}	25.57^{c}	25.08^{bc}	26.83	6.54
	C.V.	6.61	5.62	5.94	7.97		
Fruit width (mm)	Mean	23.20^{a}	23.16^{ab}	21.75^{bc}	21.21^{c}	22.33	5.91
	C.V.	3.35	4.36	6.02	7.26		
Fruit weight (g)	Mean	8.39^{ab}	9.10^{a}	7.37^{c}	7.26^{c}	8.03	14.8
	C.V.	10.61	10.66	19.54	18.73		
Pulp weight (g)	Mean	6.83^{ab}	7.26^{a}	5.88^{bc}	5.70^{c}	7.35	13.73
	C.V.	11.70	14.20	22.11	19.30		

Figures in the same letter (s) did not differ significantly at $p < 0.05$

Fruit traits showed strong to very strong positive relationships among themselves (Table 4). Fruit weight was significantly and positively correlated with fruit width (r=0.940) and fruit length (r=0.867), whereas fruit width had a strong positive correlation with fruit length (r=0.782). Fruit pulp weight had a very strong correlation with fruit weight (r = 0.986). As far as inter-class correlations of leaf and fruit traits are concerned, none of the fruit traits demonstrated a significantly strong relationship with foliar traits (Table 4). Stronger correlations between fruit weight, length, and width indicated the crucial role of these traits in deciding fruit dimensions. In addition, strong relationships between physical dimensions (length, width, and weight) and pulp weight indicate that the more fruit dimensions, the greater the pulp mass. Given that the fruit pulp of *M. dubia* is regarded as an alternate source of feedstock (Sukhadiya et al 2021) and large dimensional fruits may be a selection criteria for improvement in pulp mass, as recommended in *Tamarindus indica* by Algabal et al. (2012) and in *Trichoscypha acuminata* by Tsobeng et al. (2020).

Association between geo-climatic variables and morphological traits: The association of the geographic and climatic variables of the habitat with the morphological variability shown by individual trees in the population. To reduce the dataset of morphological variables into a meaningfully small set, principal component analysis (PCA) was done. PCA resolved the morphological variables into three PCs (Eigenvalues > 1), explaining a total variability of 80.7%. The PC 1 elucidated maximum variability (46.4 %) with positive loadings for most of the leaf characteristics except its width and negative loadings for fruit traits (Table 5). With higher loadings for leaf length and petiole length, PC 2 explained 24.0% of the total variation, while PC 3 accounted for 10.4 % of the variation with the highest loadings for leaf width. Furthermore, the strength of the correlation (person) coefficient (r) revealed that the PC1 scores had the strongest direct relationship with geo-climatic variables except for longitude (Table 6). Morphological traits (PC1) had a significant positive relationship with latitude (r = 0.725), altitude (r = 0.778), mean temperature (r = 0.639), and an inverse relationship with mean rainfall (-0.818) of the habitation. The regression equation revealed that latitude (R^2 = 0.525), altitude (R^2 = 0.605), mean temperature (R^2 = 0.408), and mean rainfall (R^2 = 0.669) explained 52.5% of the variability in PC1 scores (Fig. 3). The regression analysis further revealed that when latitude increased by 1 degree, the value of PC1 increased by 1.35 (b_1=1.3528). Similarly, every unit increase in altitude (m) and mean temperature (0 °C) increased PC1 scores by 0.06 and 0.52 times, respectively. In contrast, a reduction in PC1 scores by 0.01 with an increment of rainfall of 1 cm is explained (b_1= -0.01308) (Fig. 3).

The relationship between morphological traits and their surroundings has been advocated as the result of plants' evolutionary response to changing environmental conditions (Westoby et al., 2002). Earlier reports have confirmed the role of geo-climatic factors in the expression of dissimilarity among plant populations in terms of leaf morphology (Danquah 2010, Atchaya et al., 2019, Liu et al., 2020). In this study, latitude, altitude, and mean annual temperature showed a linear relationship with foliar morphology except, leaf width (negative vector loadings in PC1), whereas, precipitation showed an inverse relation except, leaf width (PC1). To be specific, longer (leaf length and associated characters like petiole and rachis length), narrower (leaf width), more sturdy leaves (petiole base diameter), and leaves with more leaflets and pinnae, are attributes of higher altitude and a higher MAT zone. The population inhabiting

Table 4. Intra and inter-character correlations (Pearson) matrix of 11 morphological traits in*M. dubia* populations

Characters	LL	LW	PL	RL	BD	NP/L	NL/P	FL	FW	FWt	PWt
LL	1										
LW	X	1									
PL	0.838**	X	1								
RL	0.599**	X	0.458**	1							
BD	0.669**	X	0.635**	0.432**	1						
NP/L	0.487**	-0.425**	0.471**	0.476**	0.768**	1					
NL/P	0.537**	X	0.497**	0.424**	0.595**	0.739**	1				
FL	X	X	X	X	X	-0.464**	-X	1			
FW	X	X	X	X	X	-0.439**	-0.413**	0.775**	1		
FWt	X	X	X	X	X	-0.416**	-0.446**	0.835**	0.779**	1	
PWt	X	X	X	-X	X	-0.415**	-0.450**	0.809**	0.731**	0.986**	1

Non-significant and correlations (r < 0.39) are shown with 'X',** Significant at p<0.01, * Significant at p<0.05

along the rainfall gradient, on the other hand, was wider, sturdier, and had a greater number of leaflets. In distinction to the fact that shorter and wider leaves are often at higher elevations and temperature (Guo et al., 2018), the present study uncovered a linear relation of leaf length and number of leaflets and inverse relation of width with elevation and mean annual temperature. Given that the angle of radiation is smaller at higher elevations, energy is forced to be adjacent to a larger surface area (CSE http://www.ces.fau.edu/nasa/module-3/why-does-temperature-vary/angle-of/the-sun.php).This could probably be seen as a survival strategy, during which plants evolved longer, more sturdy leaves with a greater number of leaflets so as to capture the maximum quantity of sunlight energy. In the same way, inverse relationships between rainfall and leaf traits are thought to balance water use efficiency., Liu et al. (2020) observed an inverse relation of leaf width with altitude in three species; Warren et al. (2005) noted decreased petiole length with

Table 5. Eigenvector loadings in Principal Component analysis (PCA) of 11 morphological traits of *M. dubia*. (Strong loadings in respective principal components are bold faced)

Characters	Eigenvectors		
	PC1	PC2	PC3
LL	0.259	0.417	0.287
LW	-0.184	0.091	0.758
PL	0.237	0.426	0.012
RL	0.263	0.204	0.403
BD	0.305	0.321	-0.226
NP (Pair)	0.365	0.137	-0.246
NL/P	0.348	0.131	-0.093
FL	-0.332	0.323	-0.06
FW	-0.322	0.293	-0.068
PW	-0.326	0.355	-0.157
FW	-0.326	0.374	-0.167
Eigenvalue	5.102	2.636	1.143
Proportion	0.464	0.24	0.104
Cumulative proportion	0.464	0.703	0.807

Table 6. Strength of Correlations (r) between principal components matrix of morphological traits and geo-climatic variables (Strong relations of r>0.6 are boldfaced)

Geo-climatic variables	PC1	PC2	PC3
Latitude	0.725^{**}	0.085^{NS}	-0.453^{**}
Longitude	0.090^{NS}	0.147^{NS}	-0.056^{NS}
Altitude	0.778^{**}	0.287^{NS}	-0.296^{NS}
Mean annual temperature	0.639^{**}	0.436^{**}	-0.358^{*}
Mean annual rainfall	-0.818^{**}	-0.272^{NS}	0.344^{*}

*Significant at p<0.05), ** Significant at p<0.01, NS -Non-significant

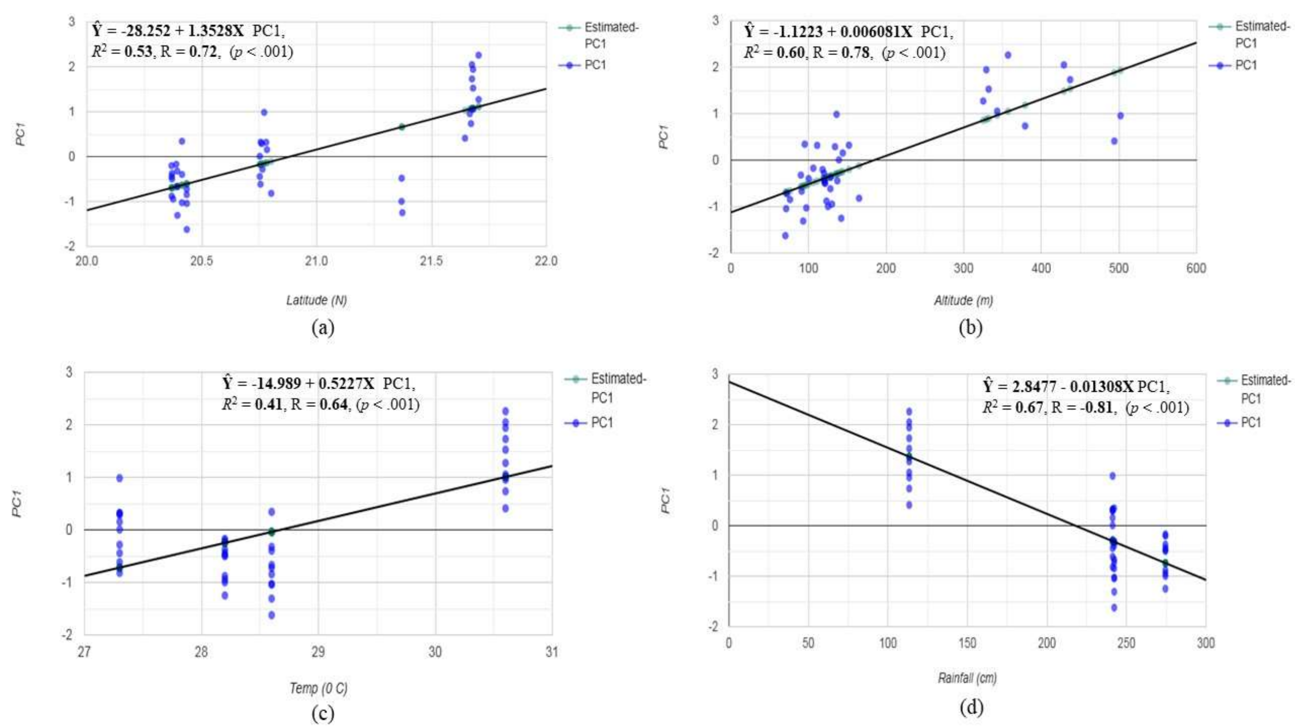

Fig. 3. Good fit plots of correlations between geo-climatic variables and PC1 scores

increasing seed source rainfall in *Eucalyptus sideroxylon;* Alcanatra-Ayala et al (2020) found longer and narrower leaves in lower rainfall regions and longer leaves in higher temperature regions in *Ternstroemia lineata.*

The relationship between fruit size (length and width) and geo-climatic gradient in PC1 (negative vector loadings) is interpreted as fruit size decreasing with latitudinal and altitudinal gradient and increasing with precipitation availability. The influence of geographic trends on fruit dimensions is also documented in *M. azedarach* by Chen et al (2015). Ramchandran and Vasudeva, (2020) noted an inverse relation between latitude and fruit length and width. Additionally, Rawat and Bakshi (2011) observed an inverse correlation of cone and seed traits with altitude and latitude in blue pine. Sudrajat et al (2016) discovered a negative relationship between fruit and seed traits and latitude and a positive relationship between fruit and seed traits and precipitation in *Anthocephalus cadamba* populations.

CONCLUSION

The study quantified the variations in foliar and fruit traits among selected natural populations of *M. dubia* in Gujarat, India. The population of *Sagai* region (*SG*) was superior for foliar traits, and the rest of the population did not significantly differ from each other. Populations from *Kaparada (KP)* and *Nanapondha (NP)* had *at par* finer fruit traits. The observed differences in morphological traits might be adaptations to geo-climatic differences. A good fit plot established that morphological variations exist among the population along latitudinal and altitudinal gradients. Furthermore, the study population's foliar and fruit traits were found to be strongly influenced by mean temperature and rainfall. Out of all the geo-climatic variables, rainfall explained the highest morphological variability in PC1, making it the most important selection pressure for shaping morphological elasticity. Moreover, the possibility of a partial role for genetic differences in phenotypic plasticity cannot be ruled out; hence, a common garden experiment is needed.

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Assessment of Low-Cost *In-Situ* Soil Moisture Conservation Measures on Soil Properties and Nutrient Availability in the Chir Pine Forests

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Abstract: The study was carried out in the subtropical chir pine forest located within the Mussoorie Forest Division, Uttarakhand, part of the Western Himalayan region. The study aimed to investigate how low-cost in-situ soil moisture conservation measures affect the soil properties and nutrient availability in chir pine forests. The ranges of pH, electrical conductivity, organic carbon, total nitrogen, available phosphorus and available potassium in the forest soil were 5.56 to 5.71; 0.055 to 0.073 mS/cm; 0.99 to 1.58%; 1.25 to 1.29%; 20.21 to 27.51 kg per ha; and 242.95 to 314.16 kg per ha, respectively. The trend of phosphorus availability for plants changed with changes in the soil pH. The maximum available phosphorus (27.51 kg per ha) was at soil pH of 5.58 in earthen bund treatments. In contrast, the minimum available phosphorus (20.21 kg per ha) was observed at 5.68 soil pH in the pine needle bund + grasses treatment. The highest available potassium was recorded in the pine needle bund treatment (314 kg per ha) at 5.56 pH and the lowest in the shallow ditch treatment (242.95 kg per ha at 5.66 soil pH. The relationship between total nitrogen percentage and organic carbon was positive. The findings highlight that soil moisture conservation treatments have the potential to enhance nutrient availability and fertility in the soil, which will ultimately promote the sustainability of chir pine forests.

Keywords: Low-cost, Soil moisture conservation, Physico-chemical properties, Management practices, Forest ecosystem

The chir pine (*Pinus roxburghii*) is a native tree species of the Himalayas and is commonly found in the Indian states of Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Darjeeling in West Bengal, and Arunachal Pradesh. In Uttarakhand, the chir pine forest contributes the highest area in the forest cover (FSI 2019). Due to the largest forest cover area, this forest acts as the biggest water catchment area for perennial rivers that originate from Uttarakhand, such as the Ganga and Yamuna. These rivers are lifelines for North Indian states.

This forest area received high annual rainfall. The two extremes of rainfall conditions, such as erosion and drought, are greatly mitigated by in-situ soil and water conservation methods (Chimdessa et al., 2019). GLASOD has recognized two types of soil degradation. The first category is soil degradation due to displacement of soil material, such as soil erosion by wind and water. The second category is in-situ soil degradation due to chemical processes like loss of nutrients and organic matter, salinisation, acidification, and pollution, and due to physical processes such as compaction, waterlogging and subsidence (Asio et al., 2009). The land degradation vulnerability covers about 25 percent of land in Asian countries and 46 percent of land in Africa, threatening about 485 million people. The degradation of over 68 percent of the land in Australia poses a severe environmental problem (Mahata and Sharma 2021).

In tropical, subtropical, and semiarid climates, water-induced soil erosion is the main cause of land degradation (Lal 2001, Bhattacharyya et al., 2015). According to the government's unified database (NAAS, 2012), water erosion accounts for 70% of the degradation of 120.7 million hectares of land (Bhattacharyya et al., 2016a). Based on the sedimentation budget across India, the gross average annual soil erosion rate is 15.6 t ha^{-1} yr^{-1}, (Majhi et al., 2021).

Soil moisture and nutrients are the most essential factors for plant growth (Vaidya et al., 2016, Ademe 2018). The proper use of soil moisture conservation structures helps to reduce the runoff rate and nutrient losses from the soil and improve the soil moisture and nutrient availability for plant growth, which in turn boosts the productivity of land and plants (Vaidya et al., 2016, Gadisa and Midega 2021). In-situ soil and water conservation measures play a significant role in alleviating the two extremes of rainfall conditions, such as erosion and drought. In temperate forest ecosystems, one of the most significant factors influencing tree growth and ecosystem function is the availability of nutrients. Nitrogen and phosphorus are the most limiting nutrients for plant growth, and their resorption is important for plant nutrient conservation (Singh and Negi 2023).

The systematic management of this forest area is essential. The nation's fundamental assets, water and soil, require wise use and conservation to sustain the growing populations of people, animals, and plants. Low-cost soil and moisture conservation measures have the potential to mitigate soil erosion, enhance water retention, and improve soil health for the sustainable management of forest ecosystems. Therefore, the present study aimed to investigate the impact of low-cost in-situ soil moisture conservation measures on soil properties and nutrient availability. Understanding soil properties and nutrient availability helps in solving the problem of lower productivity of land under chir pine forests through the application of suitable low-cost in-situ soil moisture conservation measures (SMC).

MATERIAL AND METHODS

The present experimental study was conducted in Raipur range, Mussoorie Forest Division, Uttarakhand at an elevation of 1842 m above msl with 30°21' 35.25" (N) latitude and 78°12'16.82" (E) longitude. The annual average rainfall in the region ranges from 2200 mm to 2400 mm. Total eight treatments (Table 1) were applied in the field.

From each treatment, soil samples were collected, pooled, air dried, ground, sieved in a 2 mm sieve and used for soil analysis. The soil of pH and electric conductivity (EC) were analysed using soil:water suspension in 1:2 ratio (Jackson, 1973). The organic carbon (Walkley and Black 1934), total nitrogen (Kjeldahl 1883); available phosphorus (Olsen et al., 1954), available potassium using the flame photometry (Stanford and English 1949) were estimated as per standard methods.

RESULTS AND DISCUSSION

pH: The maximum soil pH (5.71) was in T8 (control) followed by T7 (5.68), T1, T2 and the minimum soil pH (5.56) was in T5 treatment (Table 2). The near to slightly acidic in nature of soil might be due to high material transport with surface water and needle litter falling from the canopy of trees, lower accumulation of needle in control treatment releases lower acidic compounds as they decompose, which can increase the soil pH value in control treatment compared to other treatments, The soil erosion and slope combine effect may change soil pH from moderately acidic as per present study value to slightly acidic, if soil management and soil moisture conservation practice will not be adopted. Kumar et al. (2013) recorded similar soil pH values at different altitudes, i.e., 700 m (6.77 to 6.27), 800 m (6.97 to 6.536) and 1000 m (6.97 to 6.57). Ramola et al. (2020) observed that in the soil of the temperate forest of Uttarakhand, pH ranges from 5.07 to 5.87, which was acidic due to the result of podzolisation. Vaidya et al. (2016) observed that moisture conservation treatments, i.e., conservation pit (0.45 m x 0.30 m x 0.30 m), ring basin (0.6 m Radius) and half ring basin (0.6 m radius), did not significantly influence soil pH. This could potentially be attributed to a higher concentration of H^{+}, Fe^{+2}, and Al^{+3} ions in the soil, resulting in an acidic pH range across all treatments. Soil pH can be improved by different soil moisture conservation structures such as graded stone bunds (Abay et al., 2016), stone faced soil bunds and soil bunds (Worku, 2017), soil bunds and stone bunds (Kebede et al., 2011), and treated with *Sesbania* spp. and elephant grass (Tamrat et al., 2018). The range of soil pH values required for microbial activity was 5.5-8.8 (Pietri and Brookes 2008ab, Fierer and Jackson, 2006) which also regulates organic matter mineralisation, which includes carbon, nitrogen, phosphorus and sulphur mineralisation (Neina, 2019).

The phosphorus availability for plants changes with changes in the soil pH. The maximum available phosphorus (27.51 kg/ha) was at a soil pH of 5.58 in comparison to all treatments (Fig. 1). T7 treatments observed the minimum available phosphorus (20.21 kg/ha) at a soil pH of 5.68. Soil pH is a limiting factor for the availability of phosphorus in plants. Soil pH plays an important role in the transformation of P in different forms (Wang et al., 2023). The optimum soil pH range for phosphorus availability is 6.0 to 7.0. The lower soil pH in the studied soils is associated with the presence of exchangeable Al, which leads to a decrease in P solubility

Table 1. Details of applied soil moisture conservation treatments in chirpine forest

Treatments	Length (m)	Width (cm)	Depth/height
Shallow ditch- I (T1)	2 m	15 cm	10 cm depth
Shallow ditch - II (T2)	2 m	25 cm	15 cm depth
Shallow trench (T3)	2 m	40 cm top, 20 cm base	15 cm depth
Earthen bund (T4)	2 m	15 cm	15 cm height
Pine needle bund (T5)	2 m	20 cm	15 cm height
Earthen bund + Grasses (T6)	2 m	20 cm	15 cm height
Pine needle bund + Grasses (T7)	2 m	15 cm	10 cm height
Control (T8)		no soil work	

and an increase in non-labile P pools (Hou et al., 2014). At lower pH levels, phosphate tends to bind with iron or aluminum compounds in the soil, making it less accessible for plant uptake. Acidic soils inhibit the chemical reactions that release phosphorus from organic matter and minerals, resulting in lower levels of available phosphorus. The low pH and the high exchangeable acidity in soils due to high amounts of Fe and Al are responsible for P sorption (Turrión et al., 2008).

The highest available potassium was in the T5, i.e., 314 kg per ha at 5.56 pH, and the lowest in the T1 at 5.66 soil pH (Fig. 2). The relationship between the available potassium and soil acidity is difficult to predict. Kozak and Joarder (2005) reported a positive relationship between potassium and pH, but they concluded that potassium is a basic cation, although less important than calcium and magnesium, and soil acidification results from the leaching of basic cations from soils. The pH of the soil influences the release of potassium by altering the concentrations of potassium in solutions that are in equilibrium with the soil, and these concentrations are higher in acidic solutions. Semy and Singh reported that soil pH has a strong positive correlation (0.0787) with soil.

The T8 treatment recorded the highest electrical conductivity (0.073 mS/cm), followed by the T7 (0.073 mS/cm) and T4 (0.071 mS/cm) treatments, respectively. The lowest electrical conductivity (0.055 mS/cm) was in T5 treatments. The EC was non-significant among the treatments. This might be due to organic manures not containing salts and hence there was no influence of organic manure salt accumulation in soil. Soil EC was improved by different soil moisture conservation structures, such as stone faced soil bunds and soil bunds (Worku, 2017), lands treated with sesbania and elephant grass (Tamrat et al., 2018), soil bunds, integrated manure and soil bunds (Zikri et al., 2015). Vaidya et al. (2016) concluded that EC did not show significant differences due to moisture conservation methods. Acidity in soil can lead to the presence of higher concentrations of certain ions, such as hydrogen ions (H^+) and aluminum ions (Al^{3+}). These ions can compete with other nutrients like calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^+) for adsorption sites on soil particles. This can alter the ionic balance in the soil solution, affecting its electrical conductivity.

Organic carbon: The soil organic carbon was significantly influenced by different treatments; maximum (1.58%) organic carbon was observed in T4, followed by T6, T7, T1 and minimum (0.99%) was in T5 treatment in comparison to all treatments. Hailu et al. (2012) observed the highest soil organic carbon in the Fanya juu 10-year old (1.94%), followed by the Fanya juu 5-year old (1.92), compared to the control (1.68). Gebreselassie et al. (2009) also reported that higher OC in the SMC treatments (9 years old soil bund (3.17%) . In acidic soils, the breakdown of organic matter might be slower, leading to the accumulation of partially decayed organic materials. These materials can help improve soil structure, water retention, and aeration. The increase in organic carbon also increased the percentage of total nitrogen (Fig. 3). Microbial activity may drive a shift in nutrient stoichiometry, leading to an increase in the percentage of total nitrogen as organic carbon levels rise. This type of effect was observed in the T1, T3, T4, T6, and T7 treatments. The T2, T5, and T8 treatments observed a plummet in the total nitrogen percentage due to the lowering of soil organic carbon.

Plants require a lot of nitrogen (N), a soil component that,

Table 2. Physico-chemical properties of soil under different soil moisture conservation treatment applied in chirpine forest

(Mean ± SD)

Treatments	pH		EC		OC		Total Nitrogen (%)		Available P (kg/ha)		Available K (Kg per	
T1	5.66±0.11		0.070±0.014		1.40±0.40		1.28±0.03		26.89±10.94		242.95±63.3	
T2	5.62±0.17		0.068±0.016		1.35±0.36		1.26±0.03		22.74±6.63		273.84±100.1	
T3	5.62±0.09		0.065±0.015		1.26±0.43		1.29±0.04		25.91±9.32		272.94±70.7	
T4	5.58±0.17		0.071±0.011		1.58±0.60		1.28±0.04		27.51±7.88		268.80±48.1	
T5	5.56±0.21		0.055±0.007		0.99±0.24		1.25±0.05		26.25±13.81		314.16±114.2	
T6	5.58±0.13		0.065±0.009		1.48±0.50		1.29±0.03		24.46±6.05		288.77±85.9	
T7	5.68±0.15		0.073±0.014		1.42±0.45		1.27±0.03		20.21±6.78		286.91±109.8	
T8	5.71±0.23		0.073±0.015		1.08±0.22		1.26±0.06		26.00±8.57		279.72±121.9	
	F Value	Pr(>F)	F Value	Pr(>F)	F Value	Pr(>F)	F Value	Pr(>F)	F Value	Pr(>F)	F Value	Pr(>F)
	1.088	0.38	2.833	0.0115 *	3.0093	0.0079 **	1.2664	0.2792	0.802	0.589	0.546	0.797

Significance Level: 0 '***' 0.001 '**' 0.01 '*' 0.05 '

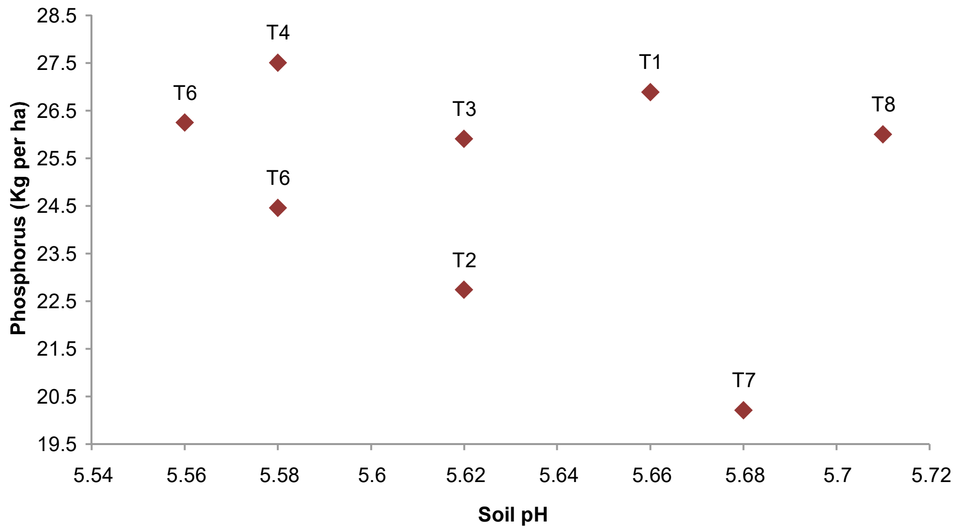

Fig. 1. Trend of nutrient availability: Phosphorus (Kg per ha) with soil pH

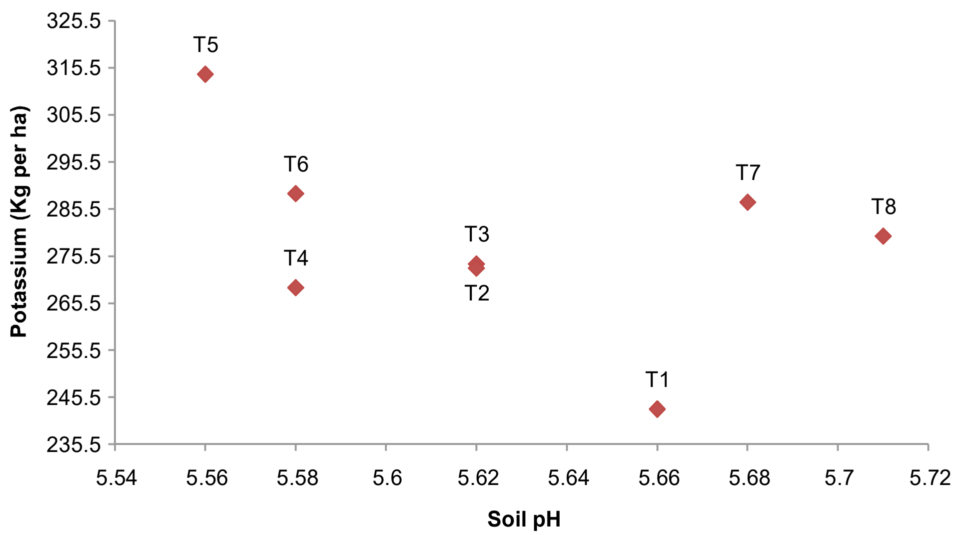

Fig. 2. Trend of nutrient availability: Potassium (Kg per ha) with soil pH

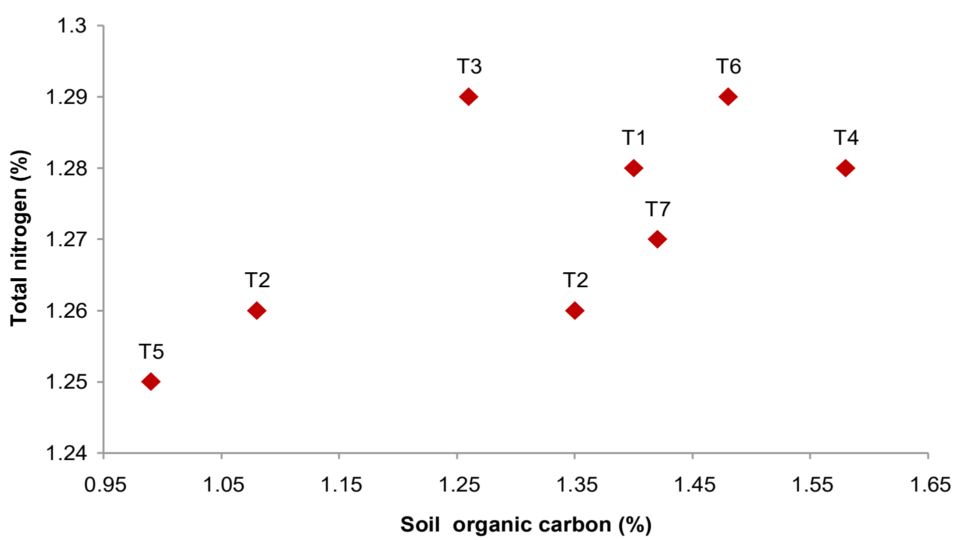

Fig. 3. Trend of nutrient availability: Organic carbon (OC%) and available nitrogen

if deficient frequently restricts plant growth. This nutrient comes from the atmosphere (78% N) and soil organic matter instead of being a constituent of minerals (rocks). Even with high dosages of N fertiliser, most plants acquire 50 to 80% of their N requirement from the soil, making soil their primary source of N. Wibowo and Kasno (2021) reported that the organic carbon and total nitrogen were strongly correlated. Tomar and Bhat (2023) recorded that nitrogen has a significant and positive correlation with organic carbon. The significant relationship between nitrogen and organic carbon, phosphorus, and potassium is similar to the findings reported by Dinesh et al. (2020).

There was no specific type of trend between available phosphorus and organic carbon (Fig. 4). There was a higher amount of available phosphorus on both sides, i.e., the lower side of OC% and the higher side of OC%. Higher amounts of available phosphorus at higher organic carbon (T4: 27.51 kg per ha @ 1.58%; T1: 26.89 kg per ha @ 1.40%) and higher amounts of available phosphorus at lower organic carbon (T5: 26.25 kg per ha @ 0.95%). Lower amounts of available phosphorus at higher organic carbon (T7: 20.21 kg per ha @ 1.40%; T6: 24.45 kg per ha @ 1.48%). There was no specific trend between available potassium and organic carbon. Among the treatments, there was a higher amount of available potassium on both sides, i.e., the lower side of OC% and the higher side of OC%. Higher amounts of available potassium at higher organic carbon (T7: 286.91 kg per ha. @ 1.42% of OC) and higher amounts of available

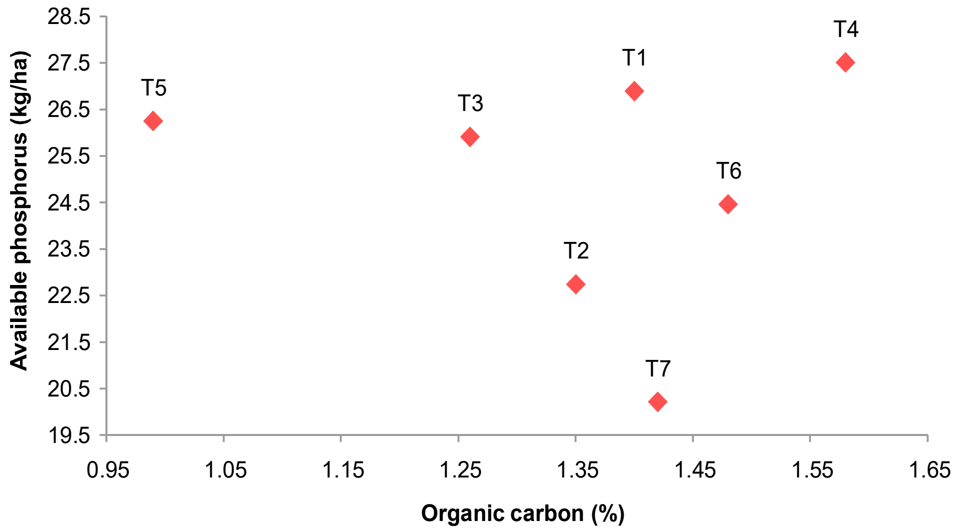

Fig. 4. Trend of nutrient availability: Organic carbon (OC%)and available phosphorus (P)

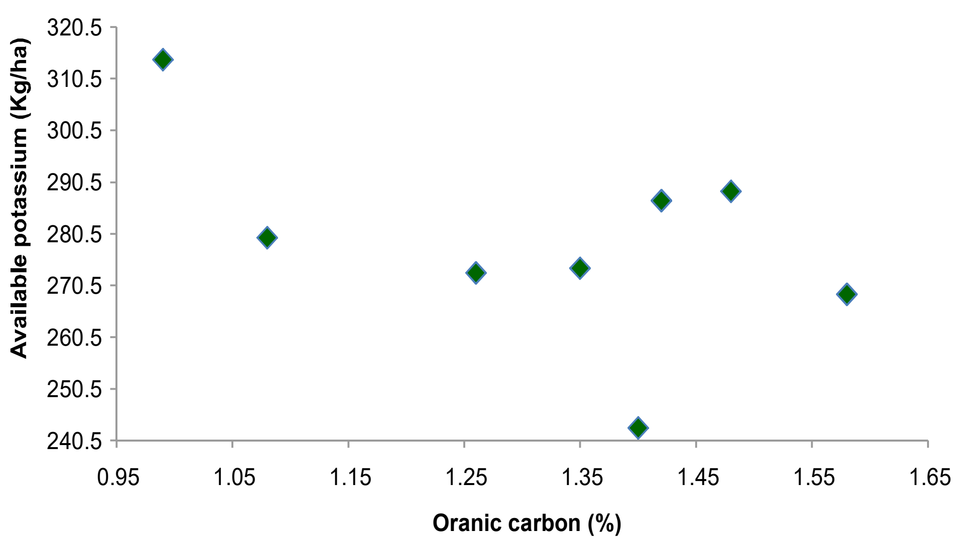

Fig. 5. Trend of nutrient availability Organic carbon (OC%) and available potassium (K)

potassium at lower organic carbon (T5: 314.16 kg per ha. @ 0.99%). Lower amount of available potassium was found at higher organic carbon (T1: 242.95 kg per ha @ 1.40% of OC).

Different treatments did not significantly influence the total nitrogen percentage. The T6 treatment recorded the highest total nitrogen percentage (1.29%), followed by T3, T7, and T2, while the T5 treatment recorded the lowest total nitrogen percentage (1.25%). Soils that are too acidic can lower the activity of microbes, which can slow down the breakdown of organic matter and the release of nitrogen from organic sources. This can lead to lower nitrogen availability for plants. Vaidya et al. (2016) observed that control treatments had less total nitrogen than the conservation pit, ring basin, and half-ring basin types of moisture conservation measures. The graded stone bund, stone faced soil bund and soil bund, level soil bund and stone bund improved total nitrogen in the soil (Abay et al., 2016, Worku 2017).

Available phosphorus: Among the different soil treatments, the maximum (27.51 kg per ha) available phosphorus was recorded in T4, followed by T1, T5 and T8 (Table 2). The trend of available phosphorus among the different SMC treatments was T7<T2 <T6 < T3< T8 < T5 < T1 < T4. Rathod et al. (2020) also observed similar trends where phosphorus ranged from 12.90 to 17.84 kg/ha in temperate forest areas. Phosphorus loss is more frequently associated with erosion and runoff. Eroded sediment carried away bound phosphorus to soil particles (Jeschke 2017). Vaidya et al. (2016) reported higher available phosphorus in the different moisture conservation measures, such as conservation pits, ring basins, and half-ring basins, than in control treatments. The stone faced soil bund and soil bund, terraces, and lands treated with *Sesbania* and elephant grass improved available phosphorus in the soil (Tadele et al., 2013, Worku 2017, Tamrat et al., 2018).

Available potassium: The T5 treatment had the highest available potassium (314.16 kg per ha), followed by T6, T7, and T8. The trend of available potassium among the different SMC treatments was T1 < T 4 < T3 < T2 < T8 < T7 < T6 < T5 in increasing order. Rathod et al. (2020) also showed a similar trend, with the potassium value ranging from 208.95 to 601.55 kg per ha. In acidic soils, phosphorus can bind to iron, aluminum, and other minerals, forming insoluble compounds. This phenomenon is known as phosphate fixation. These insoluble compounds are not easily taken up by plants and thus reduce the availability of phosphorus. Vaidya et al. (2016) reported higher available potassium in the different moisture conservation measures, such as conservation pit (ha), ring basin, and half ring basin, than in control treatments. The results of stone faced soil bund and soil bund were improved with higher available potassium in the soil (Worku 2017). Yonas et al. (2017) observed that the effectiveness of soil and water conservation significantly improved potassium than in the control SWC treatment.

CONCLUSION

The study concluded that low-cost in-situ soil moisture conservation measures within chir pine forests had significant impacts on organic carbon, electrical conductivity, soil properties, and nutrient availability. These simple and cost-effective soil moisture conservation practices significantly improve soil properties and nutrient availability. The higher values of organic carbon indicated higher amounts of organic matter in all treatment comparisons, except for the pine needle bund (T5) treatment. These practices improve soil structure, soil moisture, water-holding capacity and enhance organic carbon. This leads to enhanced nutrient availability in the soil through organic matter decomposition, which releases nitrogen, phosphorus, and potassium into the soil in forms that become available to plants over time. Additionally, the specific conservation practices used and their interactions with local soil and climate conditions will play a significant role in determining the outcomes. The findings underscore the importance of integrating such practices into forest management strategies, particularly in water-scarce regions, to ensure sustainable forest productivity and resilience. Further research could explore the long-term impacts and scalability of these measures across different forest ecosystems.

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AUTHOR'S CONTRIBUTION

RD: Conceptualization, write the original draft; Data curation, Investigation, Methodology, Formal analysis, Validation; Visualization **DK:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition, Methodology, Validation; **PK:** Conceptualization, Supervision, Writing – review & editing, Methodology

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Effect of Lime on Different Forms of Soil Acidity and Phosphorus Availability in Acid Soil

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Abstract: The incubation study was carried out in the summer of 2023 to investigate the impact of lime on various forms of soil acidity and phosphorus availability in acid soils. at Faculty of Agricultural Sciences, Siksha 'O' Anusandhan, Bhubaneswar Odisha. Red and laterite soil that were taken from the experimental field were used in two incubation investigations lab. Four amounts of lime were administered to both soils, along with two levels of phosphorus (P_0-P control & P_1-P @ 40kg/ha (0.066g/pot). In red soil the levels of lime were L_1-0.1 LR (0.2g/pot), L_2-0.2 LR (0.4g/pot), L_3-0.5 LR (1.0g/pot), In laterite soil the levels of lime were: L_1-0.1 LR (0.1g/pot), L_2-0.2 LR (0.2g/pot), L_3-0.5 LR (0.5g/pot)]. The pH of the soil increased as the lime used increased, but it decreased as the incubation times increased. After a week of incubation, the highest was P_1L_3, where 40 kg of P_2O_5 per hectare was treated in conjunction with lime application at 0.5LR. P_0L_3 showed the lowest value, with lime treatment at 0.5LR without phosphorus (6.66). Depending on the incubation period, applying varying amounts of phosphorus and lime gradually lowers the exchangeable H^+. After seven days of incubation, exchangeable H^+ in laterite soil ranged from 0.04 to 0.12 meq/100g. Following six weeks of incubation, ranged between 0.12 and 0.14 meq/100g and grew steadily. The red soil exchangeable Al^{3+} contents were considerably reduced when lime and P treatment rates increased, as well as by the combined impacts of the two applications. Following a 6-week incubation period, exchangeable $H^+ + Al^{3+}$ in red soil decreased, while the addition of varying phosphorus and lime levels resulted in an increase in exchangeable $H^+ + Al^{3+}$ in laterite soil. Following the addition of varying quantities of phosphorus and lime to red soil and laterite soil, range of organic carbon was 1.03 to 1.81% and 0.25 to 0.67% respectively. The rate of lime was when increased in both red and laterite soil, the change in available P gradually reduced. The amount of accessible calcium and magnesium also increased during 42-day incubation period.

Keywords: Lime, pH, Exchangeable H^+, Exchangeable Al^{3+}, Available P, Red soil, Laterite soil

Globally, soil acidity is acknowledged as a significant issue that has a negative impact on agricultural productivity, either directly or indirectly, particularly in temperate and tropical regions of the world (Asrat 2021). It is well recognized that soil acidity has a negative impact on nutrient availability, which in turn has a negative direct and indirect influence on crop growth. Highly worn acid soils in the tropics have low phosphorus (P) availability and a high P fixation by massive amounts of aluminium (Al) and iron (Fe) oxides (Rajneesh et al., 2018). Phosphate is applied and quickly reacts with large amounts of iron and aluminium oxides, making it less soluble in acidic soils (Bolan et al., 2003). Al oxide, which dissolves with additional acidity and eventually saturates the soils' cation exchange sites with exchangeable Al, is a typical buffer for the pH of acidic soils. Acidic soils have lesser capacity to retain water and nutrients, which results in decreased moisture and nutrient uptake as well as low biotic activity (Asrat 2021).

The phosphorus (P) is a necessary element for plant growth labile forms in soil are comparatively rare when compared to other main macronutrients (Jain et al., 2012). This is because P is naturally soluble in low solubility soil compounds and minerals. Because liming materials have such powerful acid neutralizing capabilities and may effectively eliminate existing acid, increase biological activity, and reduce toxicity of heavy metals. Lime oxides, hydroxides, carbonates, slag, and other liming materials are a few of these (Singh et al., 2023). The best way to produce crops on acid soils is to apply lime and fertilizer (phosphorus in particular). When lime is applied to the soil, Ca^{2+} and Mg^{2+} ions replace H^+, Fe^{2+}, Al^{3+}, Mn^{4+}, and Cu^{2+} ions from soil adsorption sites, raising the pH of the soil. Depending on the type of liming materials used, lime can also provide sizable amounts of Ca and Mg. Lime has indirect benefits that include better soil structure in some situations, enhanced availability of P, Mo, and B, and more favourable conditions for microbially mediated reactions including nitrification and nitrogen fixation (Nekesa et al., 2005). The current study was to assess the effect of different lime concentrations on soil pH and various types of soil acidity (pH-dependent and exchange acidity), as well as the phosphorus dynamic in acid soils treated with lime and to determine the optimum dose of lime to correct soil acidity.

MATERIAL AND METHODS

Incubation study was conducted during summer 2023 at

Faculty of Agricultural Sciences, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar Odisha to observed the effect of lime on different forms of soil acidity and phosphorus availability in acid soils. The tropical climate at the experimental site ranges from a maximum of 25.7 to 40.7˚C to low of 12.8 to 27.9˚C. In addition, the experimental site saw 10.30 mm of rainfall on average. Two distinct locations with varying pH values inside the experimental site yielded two different types of soil: red soil (pH-5.18) and laterite soil (pH-5.78). There were eight treatments made up the factorial complete randomized design with three replications. Following crop harvest, soil samples were taken from the experimental field, allowed to air dry, and then sieved through a 2 mm mesh sieve before being stored for analysis. In each container, 750 g of soil samples were collected. Three levels of lime (0.1, 0.2, and 0.5 LR) and two levels of phosphorus (P_0-no phosphorus & P_1-P_2O_5 @ 40 kg per hectare) were given to both soils in accordance with the treatment details. Pure $CaCO_3$ lime was used. The beakers' mouths were open, and they were set up like labs. Every two days, the weight of the beakers was recorded, and the soil's field capacity was maintained by adding an appropriate amount of distilled water to offset moisture loss. Sample of the soil was taken from each pot every seven days, i.e., after seven, fourteen, twenty-one, thirty, and forty-two days. On February 24, 2023, red soil was collected, and on June 6, 2023, laterite soil was obtained. The soil was kept in a laboratory setting for a period of six weeks. Using accepted techniques, the different physico-chemical characteristics of the experimental soil, such as its texture, pH, organic carbon, available phosphorus, exchangeable acidity, available calcium, and magnesium, were ascertained. The analysis was done by DMRT method by SPSS software.

RESULTS AND DISCUSSION

Soil pH: Soil pH increased as lime treatment levels rose (Table 1), but it decreased as incubation times increased. pH range in red soil was 5.8 to 7.12. Following a week of incubation, the highest was in P_0L_3, where lime was treated at 0.5LR with no phosphorus level, followed by P_1L_3 (where 40kg of P_2O_5 per hectare was applied at 0.5LR,) and the lowest value was in the control. After an incubation period of 42 days, the same patterns persisted. The application 0.5LR of lime increased the soil pH as compared to other levels of lime i.e., 0.1 LR & 0.2 LR.

The pH range in laterite soil was 5.56 to 6.69. After a week of incubation, the highest was in P_1L_3, where 40 kg of P_2O_5 per hectare was treated in conjunction with lime application at 0.5LR. P_0L_3 showed the lowest value, with lime treatment at 0.5LR without phosphorus. Following 42 days of incubation, the highest was in P_0L_3, where lime was applied at 0.5LR without any phosphorus. This was followed by P_1L_3, where lime was applied at 0.5LR with 40kg P_2O_5 per hectare, and P_0L_1 showed the lowest value. When lime and phosphorus were not added, the control group did not experience any notable alterations.

Limes needed to neutralize harmful components in the soil and raise the pH of acidic soil. The pH scale is used to represent soil reactivity and determine if the soil is neutral, acidic, or alkaline. The molar activity, or concentration, of hydrogen ions in the soil solution is measured by soil pH (Moody and Cong 2008). The pH of the soil can be used to determine the kind of chemical reactions that are probably occurring there. It influences microbial activity, root growth, and the availability and toxicity of nutrients. Liming is done to raise the pH to the range of 5.5 to 6.5, which is ideal for most plant growth. Liming is one of the management techniques for managing soil fertility since it lowers the acidity of the soil.

Exchangeable H^+: Depending on the incubation period, applying varying amounts of phosphorus and lime gradually lowers the exchangeable H^+ (Table 2). After seven days of incubation, the exchangeable H^+ in red soil ranges from 0.04 to 0.12 meq/100g. The highest exchangeable H^+ was in P_1L_0, where no lime was applied along with 40kg P_2O_5 per hectare. The exchangeable H^+ ion concentration was observed to be steadily declining after 6 weeks of incubation periods.

After seven days of incubation, exchangeable H^+ in laterite soil ranged from 0.04 to 0.12 meq/100g. The P_0L_2 had

Table 1. Effect of different levels of lime and phosphorus on soil pH (days after incubation)

Treatment	Red soil		Laterite soil	
	7	42	7	42
	pH	pH	pH	pH
P_0L_0	5.8	5.3	5.5	6.22
P_0L_1	5.98	5.74	6.5	6.14
P_0L_2	6.31	5.8	6.63	6.44
P_0L_3	7.12	6.31	6.66	6.58
P_1L_0	5.81	5.28	6.08	6.43
P_1L_1	5.9	5.56	6.39	6.25
P_1L_2	6.24	5.7	6.4	6.31
P_1L_3	6.37	6.28	6.69	6.51
CD (p=0.05)				
P	NS	NS	NS	0.091
L	0.443	0.263	0.256	0.129
P × L	NS	NS	0.362	0.183

P_0 and P_1 are no phosphorus and 40kg P_2O_5 per hectare respectively, L_0, L_1, L_2 and L_3 are no lime, 0.1 LR, 0.2 LR, 0.5 LR respectively the exchangeable H^+ when no phosphorus and lime @0.2LR were treated, while the P_1L_0 area had the lowest, when no lime and 40kg P_2O_5 per hectare were applied. Following six weeks of incubation, the concentration of Exchangeable H+ ions varied from 0.12 to 0.14 meq/100g and was progressively elevated. Lime dissociates into $Ca^{2+}$and OH^- ions when it is applied to acid soils with H^+ concentrations. The pH of the soil solution will rise as a result of the hydroxyl ions' reaction with the hydrogen-forming water (Buni 2014).

Exchangeable Al^{3+}: The red soil's exchangeable Al^{3+} concentrations were considerably reduced when lime and P application increased, along with the combined impacts of the two applications. The exchangeable Al^{3+} in red soil ranged from 0.02 to 0.06 meq/100g. After incubation for seven days, P_1L_2 had the highest exchangeable Al^{3+}, followed by P_0L_2 and the control with the lowest value. It the exchangeable Al^{3+} had nearly completely disappeared after 6 weeks of incubation. The exchangeable aluminum content of the soil was significantly decreased by P's interaction with lime (Opala et al., 2018). When combined with 40kg of P_2O_5 per hectare, the entire dose of lime (0.5 LR) needed to neutralize the soil considerably decreased the amount of soil exchangeable Al to nearly zero (Table 3).

Exchangeable Al^{3+} levels in laterite soil were also shown to be considerably reduced with increased lime and P application rates as well as by the interaction effects of lime and P application. Exchangeable Al^{3+} was ranged from 0.001 to 0.02 meq/100g. When no phosphorus and lime @0.2LR were administered, the maximum value of exchangeable Al^{3+} was obtained in P_0L_2, while the lowest value in P_1L_3, when 40 kg P_2O_5 per hectare and lime @ 0.5 LR were applied after 7 days of incubation. The exchangeable Al^{3+} had nearly completely disappeared after 6 weeks of incubation. Exchangeable Al^{3+}increased with addition of different levels of phosphorus and lime levels but declined with increasing incubation periods (Table 3).

Table 2. Effect of different levels of lime and phosphorus on exchangeable H^+ (days after incubation)

Treatment	Red soil		Laterite soil	
	7	42	7	42
	H^+	H^+	H^+	H^+
P_0L_0	0.08	0.05	0.1	0.12
P_0L_1	0.04	0.04	0.11	0.12
P_0L_2	0.04	0.02	0.12	0.14
P_0L_3	0.04	0.02	0.1	0.13
P_1L_0	0.12	0.05	0.04	0.12
P_1L_1	0.1	0.04	0.08	0.13
P_1L_2	0.07	0.03	0.06	0.13
P_1L_3	0.08	0.01	0.08	0.14
CD (p=0.05)				
P	0.021	NS	0.030	NS
L	0.030	0.013	NS	NS
P × L	NS	NS	NS	NS

Table 3. Effect of different levels of lime and phosphorus on exchangeable Al^{3+} (days after incubation)

Treatment	Red soil		Laterite soil	
	7	42	7	42
P_0L_0	0.02	0.01	0.01	0.00
P_0L_1	0.04	0.01	0.01	0.00
P_0L_2	0.05	0.01	0.02	0.00
P_0L_3	0.04	0	0.01	0.00
P_1L_0	0.03	0	0.01	0.00
P_1L_1	0.04	0	0.008	0.00
P_1L_2	0.06	0	0.005	0.00
P_1L_3	0.04	0	0.001	0.00
CD (p=0.05)				
P	NS	NS	NS	NS
L	0.019	NS	NS	NS
P × L	NS	NS	NS	NS

The exchangeable acidity in soils is caused by Al^{3+} ions. This is because, in soils that are moderately to strongly acidic, only Al^{3+} is a common exchangeable cation (Bohn et al., 2001). Achalu et al. (2012) observed that applying lime at a rate of 10 tons per hectare reduced the soil's exchangeable acidity by approximately 90.7%, from 2.80 cmol (+) kg in the control to 0.26 cmol (+) kg. Temesgen *et al.* (2017), observed that lime application rates increased, exchangeable acidity and Al^{3+} dropped significantly. Because lime-added CO_3^{2-} surfaces reduce active acidity by consuming H+ in soil solution, Al is exchanged for added base cations (mostly lime-borne divalent alkaline earths Ca and Mg) and precipitates as insoluble $Al(OH)_3$ due to its dramatically reduced solubility in circum neutral pH values (Antoniadis et al., 2015).

The red soil's exchangeable H^+ + Al^{3+} levels were considerably reduced when lime and P application rates increased, along with the combined impacts of the two applications. Exchangeable $H^+ + Al^{3+}$ranged from 0.08 to 0.16 meq/100g in red soil. After incubation for seven days, P_1L_0 (i.e., 0.16 meq/100g) had the highest value of exchangeable H^+ + Al^{3+},where no lime and 40 kg P_2O_5 per hectare were applied. P_1L_1 (0.14 meq/100g) had the lowest value, with no phosphorus and lime @0.1LR applied, and P_0L_3 (0.08 meq/100g) had the highest value. The exchangeable $H^+ + Al^{3+}$ decreased after six weeks of incubation. The interaction of lime by P highly reduced the exchangeable $H^+ + Al^{3+}$ content of the soil (Opala et al., 2018).

Exchangeable $H^+ + Al^{3+}$ levels in laterite soil were also considerably elevated with increasing lime and P application rates as well as by the interaction effects of lime and P application. Exchangeable $H^+ + Al^{3+}$ ranged from 0.05 to 0.13 meq/100g. When no phosphorus and lime @0.2LR were applied, the maximum value of exchangeable Al^{3+} was in P_0L_2 (0.13 meq/100g), while the lowest was in P_1L_0 (i.e., 0.05 meq/100g), where, after 7 days of incubation, 40kg P_2O_5 per hectare was applied along with no lime. Following six weeks of incubation, adding varying amounts of lime and phosphorus led to an increase in exchangeable $H^+ + Al^{3+}$ (Table 4). Highest was in P_0L_2 (i.e., 0.14 meq/100g) which was at par with P_1L_3.

Available phosphorus: As application of lime increased, the change in available P gradually decreased (Table 5). After seven days of incubation, the amount of accessible phosphorus in red soil ranged from 25.67 to 88 kg ha^{-1}. P_1L_3 had the highest, 88 kg ha^{-1}, when lime @0.5LR and 40-kilogram P_2O_5 per hectare were administered. P_1L_2 had the second-highest, 85 kg ha-1, when lime @0.2LR and 40 kg P_2O_5 per hectare were applied. The control group, which received neither lime nor phosphorus, had the lowest. Following 42 days of incubation, the highest 63.12 kg $ha^{-1}$was in P_1L_2, while the lowest 24.4 kg $ha^{-1}$was in P_0L_1. This suggests that phosphorus availability increases when lime is applied alone, but decreases when both lime and phosphorus are added.

After 7 days of incubation, the amount of accessible phosphorus in laterite soil ranged from 34.33 to 80.8 kg ha^{-1}. The lowest was in control, or no phosphorus and lime were applied. After incubation for 42 days, the highest was in P_1L_3 or 68.2 kg ha^{-1}, while the lowest was in P_0L_2, where lime @0.2LR was applied in addition to no phosphorus. This suggests that phosphorus availability increases when lime is applied alone, but availability decreases where both lime and phosphorus were added.

Penn and Camberato (2019), observed that fixing of P by Ca and adsorption of P to Fe and Al oxide surfaces are likely the main causes of the decrease in soil accessible P following lime application, rather than pH changes in the soil. In order to improve the release of phosphate ions fixed by Al and Fe ions into the soil solution, liming acidic soils may raise the pH of the soil. Achalu et al. (2012) concluded that acid soil can be limed to raise the pH above 6 and remedy the P shortage. Lime application increases accessible P because it improves soil acidity, which increases P availability (Kisinyo 2016). The small quantity of fixed P was released into the soil by lime, making it available for the crop. In order to hydrolyze the Al and Fe ions that accumulated with P, agricultural liming materials are a lucrative soil addition (Kiflu et al., 2017). As a result, the phosphate ion that precipitated was released into the soil solution and became available for plant absorption. By promoting the mineralization of soil organic phosphorus, liming can raise the availability of phosphate (Ameyu et al., 2019).

Available calcium:. After 7 days of incubation, accessible calcium varied from 0.094 to 0.112% (Table 6). The highest was found in P_1L_3, or 0.112%, where 40 kg of P_2O_5 and lime at 0.5LR were administered. The lowest was in control, or 0.094%, where neither phosphorus nor lime were treated.

Table 4. Effect of different levels of lime and phosphorus on exchangeable acidity ($H^+ + Al^{3+}$) (days after incubation)

Treatment	Red soil		Laterite soil	
	7	42	7	42
P_0L_0	0.1	0.06	0.12	0.12
P_0L_1	0.1	0.05	0.12	0.12
P_0L_2	0.09	0.03	0.13	0.14
P_0L_3	0.08	0.02	0.1	0.13
P_1L_0	0.16	0.06	0.05	0.12
P_1L_1	0.14	0.04	0.08	0.13
P_1L_2	0.13	0.03	0.06	0.13
P_1L_3	0.12	0.02	0.08	0.14
CD (p=0.05)				
P	0.020	NS	0.030	NS
L	NS	0.024	NS	NS
P × L	NS	NS	NS	NS

Table 5. Effect of different levels of lime and phosphorus on Avail P (kg/ha) (days after incubation)

Treatment	Red soil		Laterite soil	
	7	42	7	42
P_0L_0	25.67	29.19	34.33	39.5
P_0L_1	29.01	24.4	35.5	39.64
P_0L_2	30.36	30.17	37.8	39.28
P_0L_3	35	34.12	40.64	39.45
P_1L_0	66.91	27.4	52.1	49.93
P_1L_1	75	37	41	66.15
P_1L_2	85	63.12	75.43	52.65
P_1L_3	88	32.61	80.8	68.2
CD (p=0.05)				
P	9.233	7.319	4.092	4.759
L	NS	10.350	5.786	6.730
P × L	NS	14.638	8.183	9.518

Table 6. Effect of different levels of lime and phosphorus on avail Ca & Mg (%) (days after incubation)

Treatment	Red soil				Laterite soil			
	7		42		7		42	
	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg
P_0L_0	0.094	0.024	0.112	0.001	0.082	0.045	0.124	0.067
P_0L_1	0.096	0.024	0.125	0.004	0.078	0.054	0.132	0.069
P_0L_2	0.108	0.024	0.122	0.004	0.077	0.067	0.14	0.063
P_0L_3	0.109	0.026	0.12	0.002	0.07	0.06	0.145	0.08
P_1L_0	0.096	0.024	0.116	0.004	0.074	0.081	0.08	0.074
P_1L_1	0.104	0.021	0.129	0.003	0.078	0.076	0.092	0.104
P_1L_2	0.109	0.026	0.12	0.001	0.077	0.077	0.085	0.076
P_1L_3	0.112	0.027	0.154	0.006	0.085	0.074	0.088	0.072
CD (p=0.05)								
P	NS	NS	0.008	0.001	NS	0.009	0.014	0.004
L	0.012	NS	0.011	0.001	NS	NS	NS	0.006
P × L	NS	NS	0.015	0.002	0.010	NS	NS	0.008

Following 42 days of incubation, the accessible calcium content increased in accordance with the incubation duration and same pattern was followed, with P_1L_3 exhibiting the highest 0.154 percent and the control group exhibiting the lowest 0.112%.

After seven days of incubation, accessible calcium in laterite soil ranged from 0.070 to 0.085%. The highest was in P_1L_3, where 40 kg of P_2O_5 and lime @ 0.5LR were applied, and the lowest was recorded in P_0L_3, where neither phosphorus nor lime @ 0.5LR were administered. Following 42 days of incubation, the accessible calcium content increased in accordance with the incubation time. The maximum was, 0.145%, in P_0L_3, while the lowest of 0.112%, was in P_1L_0, where no lime was applied with 40 kg of P_2O_5 per hectare.

Acidic cations such as H, Al, and Fe replace base cations on exchange sites and in the soil solution when base cations are removed, particularly by leaching and erosion (Johnston, 2004). Extremely low amounts of exchangeable calcium are in highly worn tropical soils, such as Oxisols; crops grown on these soils show signs of calcium insufficiency when exchangeable calcium is less than 1 cmol kg^{-1}. Soil exchangeable calcium (Ca) and magnesium (Mg) can be increased by applying limestone (calcium carbonate) or dolomatic lime (Ca and Mg bicarbonate). Achalu et al. (2012) observed that application of lime neutralizes part of the acidity of the soil, releasing negative charges that are subsequently filled by basic cations in the soil exchange complex. The applied lime increases the concentration of Ca^{2+} and raises the pH of the soil by dissociating agricultural lime and replacing H^+ and Al^{3+} in the soil solution and soil exchange complex. This explains the direct relationships observed between pH, exchangeable Ca^{2+}, and CEC with increasing lime rates. Similar to this, the presence of pH-dependent negative charges, which might rise in response to an increase in soil pH brought on by the application of agricultural lime, may be the cause of the direct correlation between CEC and pH. (Ameyu 2019)

Available magnesium: After seven days of incubation, accessible magnesium varied from 0.024 to 0.027% (in P_1L_3, 40 kg of P_2O_5 and lime at 0.5LR were applied. Following 42 days of incubation, available magnesium content increased to 0.068%, in P_1L_0, where no lime was applied along with 40 kg P_2O_5 per hectare, and the lowest was 0.041%, in P_1L_1, where 40 kg P_2O_5 per hectare was applied along with lime @0.1LR (Table 6). After seven days of incubation, accessible magnesium in laterite soil varied from 0.045 control to 0,081%. P_1L_0T. Following 42 days of incubation, the available magnesium content increased in. The highest was 0.104%, in P_1L_1, where 40 kg of P_2O_5 per hectare was applied along with lime @0.1LR, and the lowest value, was f in P_0L_2, where no phosphorus was applied along with lime @0.2LR.

Acidic cations such as H, Al, and Fe replace base cations, particularly Mg, that are removed from exchange sites and the soil solution through leaching and erosion (Johnston, 2004). The availability of orthophosphate ($H_2PO_4^-$), nitrate (NO_3^-), and sulfate (SO_4^{2-}) anions to plant roots and the activities of exchangeable basic (Ca^{2+}, Mg^{2+}, and K^+) cations with soil organic matter content may be hindered by acidifying ions. Soil exchangeable calcium (Ca) and magnesium (Mg) can be increased by applying limestone (calcium carbonate) or dolomatic lime (Ca and Mg bicarbonate) Achalu et al. (2012), observed that when lime is applied to neutralize some of the acidity of the soil, the negative charges within the soil exchange complex are released, allowing basic cation.

CONCLUSION

Extremely acidic soils require limes and fertilizers, especially in phosphorus-deficient soils. Lime combined with the least amount of chemical fertilizer specifically, phosphorus is thought to be a more affordable, environmentally friendly, and long-lasting option than chemical fertilizer alone. This applying varying amounts of phosphorus and lime steadily reduced the exchangeable H^+ and exchangeable Al^{3+} over the course of the incubation time. It was shown that applying varying amounts of phosphorus and lime to an exchangeable acidity gradually reduces its value in accordance with the incubation duration. When lime is the only material applied, phosphorus availability rises; when both lime and phosphorus are treated, availability falls. An efficient way to mobilize and make more phosphorus available for plant uptake and soil pH management, which further affects nutrient availability, is to add lime to acidic soils. Different lime and phosphorus applications greatly raised the pH and accessible P of the soil, but they also dramatically reduced the exchangeable acidity of acidic soils.

AUTHORS CONTRIBUTION

G. Sahu conceived and designed, performed data analysis, and wrote the initial draft of the manuscript. D. Panda conducted experiments, collected data, and contributed to the interpretation of results. B. Bhuyan provided critical revisions, assisted in methodology design, and supervised the research. S. Mishra contributed to writing the manuscript, created visualizations, and coordinated submission processes. All authors read and approved the final manuscript.

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Effect of Nitrogen Management Practices in Conjunction with Farmyard Manure on Soil Properties and Fruit Yield of Chilli (*Capsicum annuum* L.)

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Abstract: Nitrogen (N) management practices has a significant impact on soil sustainability and chilli fruit yield. Therefore, an experiment study was conducted at PAU, Ludhiana during 2022-23 in split-plot design with three replicates. There are two main plots *i.e.* without and with farmyard manure (FYM) and thirteen N treatments in sub plots *i.e.* 4 different N-fertilizer levels: 0, 75, 113 and 150 kg N/ ha applied in two, three, four and five equal split doses. The results revealed that INM plots *i.e.* integration of N-fertilizer practices in conjuction with FYM @ 25 t/ha result siginifiantly higher fruit yield along with improving the soil properties than N-fertilizer practices without FYM. The treatment 113 kg N/ha applied in 4 split doses has significantly higher fruit yield (45.8%) along with higher macro and micro-nutrients with respect to recommended dose of fertilizer treatment 75 kg N/ha applied in two split doses. Only two split N-fertilizer doses were not ensured the sustainable crop performance. Therefore, 113 kg N/ha applied in four equal split doses integrated with FYM @ 25 t/ha in chilli would be beneficial for sustainable fruit production and soil fertility.

Keywords: Chilli, Farmyard manure, Fruit yield, Nitrogen management, Soil fertility

Chilli (*Capsicum annuum* L.) is one of the most significant spice crop grown for their aromatic fruit which is used for pungency, spice, taste and culinary purposes (Naz et al., 2006). It is a rich source of vitamins, capsaicin alkaloid, protein, and minerals such as iron, copper, phosphorus and calcium (Prathibha et al., 2013). It is prominent for its medicinal effects, therapeutic uses and nutritional value in addition to its use as flavouring agent in food-based industry (Deepa et al., 2007). India is largest producer, consumer and exporter of chillies in world followed by Bangladesh, China and Thailand. Andhra Pradesh is largest producer of chilli in India contributing 30% of total area (Kumar et al., 2016). In Punjab, evolution of chilli hybrids has boosted the production and area under vegetables growers and occupies an area of 8.78 thousand ha with a production of 17.63 thousand tonnes and productivity of 2.01 t/ha (Dhaliwal et al., 2015).

Nitrogen (N) plays the most significant role in increasing the metabolic and physiological exertion of plants. It is a structural component of proteins, plant physiology, fruit yield and photosynthesis assimilation. Most of the Indian soils are deficient in N element (Rekha et al., 2018). The excessive application of chemical N-fertilizer cannot sustain soil fertility and optimal fruit yield in highly intensive agricultural systems (Powlson et al., 2011). The widespread use of chemical N-fertilizers and the limited use of farmyard manure (FYM) in modern agriculture are resulting in loss of soil organic carbon (SOC) and multi-nutrient deficiencies. India consumed 16% out of total N-fertlizer globally due to knowledge gap between farmers and scientists and availability of higher subsidized N-fertilizers. The trends of N consumption have increased substantially in India which can leads to nitrate contamination of surface and groundwater (Bijay-Singh and Craswell 2021). Currently, agriculture specialists suggest farmers to shift their mindsets towards utilizing Integrated Nutrient Management (INM) approach to replace a portion of inorganic N-fertilizers with more sustainable and eco-friendly sources of nutrients. The FYM is an eco-friendly practice of INM approach which helps in improving the slow decomposition of nutrients. It can help in sustaining the productivity by conservation of organic N to available form and hence improve the soil fertility along with fruit yield. The conjoint integration of FYM with chemical N-fertilizer helps to improve the fruit productivity and agricultural sustainability, whereas sole application of N-fertilizer degrades the soil fertility. The INM can improve the microbial decomposition of organic matter which helped in conversion of organic N to plant available form (Sinha et al., 2017).

Nitrogen management techniques such as optimizing the distanct timing and levels of N-fertilizer application have proven to be effective in increasing nitrogen use efficiency, yield and reducing nutrient losses such as leaching, denitrification, etc (Shafeek et al., 2012). The fruit yield of chilli reduced in present RDF treatment *i.e.* 75 kg N/ha applied in two split doses due to drastic reduction in fruit yield at later pickings (Babanjeet et al., 2022). Limited information is available related to the effect of timing and levels of N application with and without FYM on soil properties along with fruit yield in chilli. Therefore, the present investigation has been planned to investigate for enhancing the chilli productivity while maintaining the organic carbon stocks under different N management practices.

MATERIAL AND METHODS

The present study was conducted on chilli crop at Research Area, Department of Soil Science, Punjab Agricultural University, Ludhiana, Punjab, India during the summer season of 2022-23. The research area has a semi-arid and subtropical climate which represents the Indo-Gangetic alluvial plains in north-western India. The area is situated at 247 m AMSL with 30°55' N latitude and 75°49'E longitude. It contributes around 76% of total rainfall obtained (660 mm) during the period of July-September. The agro-metereological data (Fig. 1) recorded during the crop period (March-August) showed that the average maximum weekly temperature fluctuates between 32.8°C and 43.7°C and average minimum weekly temperature fluctuates between 17.1°C and 29.0°C. Further, maximum evaporation occurs at 23rd week (5-11 June) and maximum rainfall occurs at 29th week (17-23 July), respectively. The overall 452.5 mm of rainfall was recorded throughout the crop period.

Experimental methodology: The soils of the experimental field was sandy-loam in texture, optimal *p*H and EC, low in available nitrogen, medium in available phosphorus and potassium. The chilli (*Capsicum annuum* L.) crop was sown in nursery on 13th November, 2021 and transpanting was done on 13th March, 2022, respectively. Farmyard manure was applied at the rate of 25 t/ha as per recommended practices for vegetable crop in Punjab, PAU, Ludhiana. The N-fertilizer was applied through drilling at transplanting and top dressing at every picking. The P-fertilizer at the rate of 30 kg P_20_5/ha was drilled through SSP (16% P_2O_5) and K-fertilizer at the rate of 30 kg K_2O/ha was drilled through MOP (60% K_2O) before chilli transplanting. The study was undertaken to integrate N-fertilizer management practices with and without FYM on soil properties along with fruit yield. The treatments details are given in Table 1. The crop was planted on ridges at 75 cm apart with plant to plant spacing of 45 cm. About 0.5 grams of composite soil samples was taken before and after harvesting of crop and analyzed in soil testing laboratory by following the standard methods (Table 2 and 3). The fruit yield (q/ha) was recorded from each plot at every picking when chilli turns colour from green to red. The experimental results were statistical analyzed using CPCS-1 software given by Cheema and Singh (1991). Mean comparisions were carried out to compare the means at (p = 0.05).

RESULTS AND DISCUSSION

Soil Chemical Parameters

Soil *p*H, electrical conductivity and soil organic carbon: There were significant variation between the without and with FYM for soil *p*H. The initial values of the experimental field were depicted in Table 3. The results depicted in Table 4 showed that FYM treated plots have a lower *p*H value (7.24) than without FYM plots (7.32). The T_1 treatment (0 kg N/ha) recorded the highest *p*H value (7.33). The decrease in surface soil *p*H with the integrated application of N-fertilizer with FYM may be attributed to the release of CO_2 and the production of organic acid during the mineralization and persistent decomposition of FYM (Singh et al., 2015). Rajneesh et al., (2017) revealed that an increase in N-

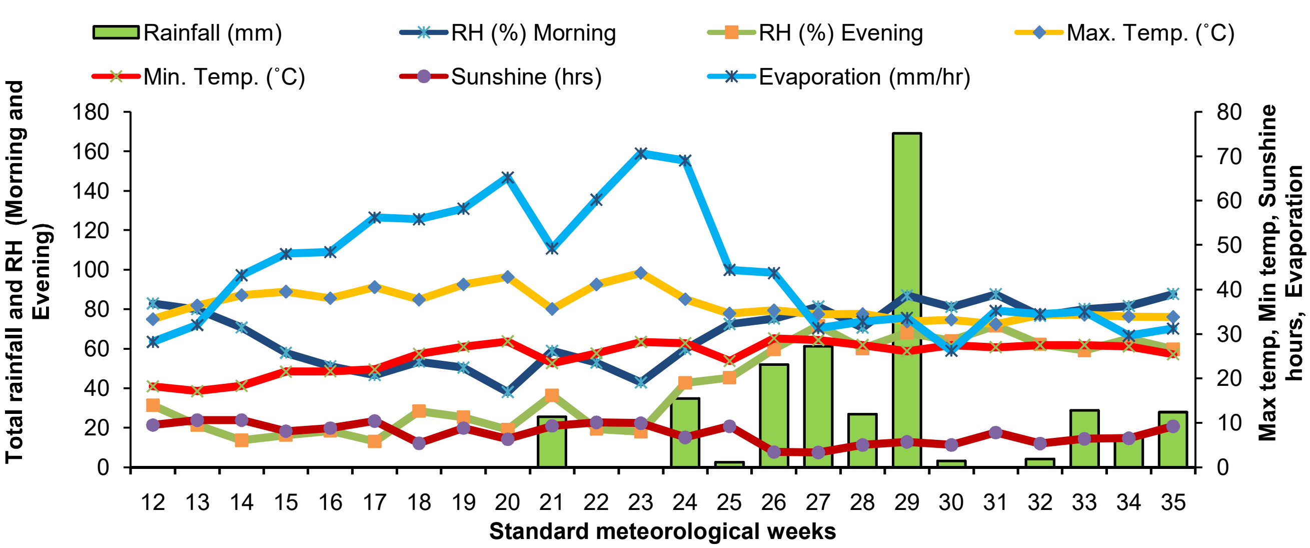

Fig. 1. Weekly mean meteorological data recorded during2022-23 at PAU, Ludhiana

fertilizer levels can decrease the soil *p*H due to the release of H^+ ions and hydrolysis of inorganic urea. The decrease in surface soil *p*H has a positive influence on the availability of different nutrients, notable Zn, Mn, Fe and P (Benbi et al., 2012). The N-fertilized plots without FYM plots have higher EC value (0.18 dS/m) than FYM plots (0.15 dS/m). There was a significant decrease in EC range with an increase in the level of N-fertilizer application (Table 4). The higher EC value (0.20 dS/m) was observed in the T_1 treatment and the lowest EC (0.15 dS/m) was attained in T_{11}, T_{12} and T_{13} treatments by substitution of 150 kg N/ha in three, four and five equal splits, respectively. The lower EC values under organic amendment (FYM) could be attributed to increased soil water holding capacity because of improved soil aggregation (Duhan and Singh 2002). Soil organic carbon is the most imperative indicator of agricultural sustainability and soil health. The treatments T_4, T_8, T_{11} and T_{12} have maximum SOC (0.43%) due to higher build-up of SOC due to slower and less persistent decomposition rate of FYM because of polyphenol

Table 3. Basic initial soil properties (0-15 cm soil profile)

Soil parameters	Initial values
Sand (%)	74.5
Silt (%)	16.3
Clay (%)	9.2
Textural class	Sandy-loam
Bulk density (g/cm^3)	1.62
Particle density (g/cm^3)	2.61
Soil *p*H	7.40
Electrical conductivity dS/m at 25^{0}C	0.23
Soil organic carbon (%)	0.41
Available nitrogen (kg/ha)	137.6
Available phosphorus (kg/ha)	18.4
Available potassium (kg/ha)	127.6
DTPA extractable zinc (mg/kg)	2.59
DTPA extractable copper (mg/kg)	0.35
DTPA extractable iron (mg/kg)	11.7
DTPA extractable manganese (mg/kg)	3.71

Table 1. Treatments details

S. No.	Main plots (2)
F_0	Without FYM
F_{25}	With FYM @ 25 t/ha (RDF)
	Sub plots (13)
T_1	0 kg N/ha (Control)
T_2	75 kg N/ha application in 2 equal splits at transplanting and after 1st picking (RDF)
T_3	75 kg N/ha application in 3 equal splits at transplanting, after 1st and 2nd picking
T_4	75 kg N/ha application in 4 equal splits at transplanting, after 1st, 2nd and 3rd picking
T_5	75 kg N/ha application in 5 equal splits at transplanting, after 1st, 2nd, 3rd and 4th picking
T_6	113 kg N/ha application in 2 equal splits at transplanting and after 1st picking
T_7	113 kg N/ha application in 3 equal splits at transplanting, after 1st and 2nd picking
T_8	113 kg N/ha application in 4 equal splits at transplanting, after 1st, 2nd and 3rd picking
T_9	113 kg N/ha application in 5 equal splits at transplanting, after 1st, 2nd, 3rd and 4th picking
T_{10}	150 kg N/ha application in 2 equal splits at transplanting and after 1st picking
T_{11}	150 kg N/ha application in 3 equal splits at transplanting, after 1st and 2nd picking
T_{12}	150 kg N/ha application in 4 equal splits at transplanting, after 1st, 2nd and 3rd picking
T_{13}	150 kg N/ha application in 5 equal splits at transplanting, after 1st, 2nd, 3rd and 4th picking

Table 2. Standard analytical methods followed in soil analysis (0-15 cm soil profile)

Soil properties	Standard estimation method	References
*p*H (1:2 soil: water suspension)	Glass membrane electrode using *p*H meter	Jackson (1973)
Electrical conductivity (1:2soil: water suspension)	Potentiometric method using EC Solubridge meter	Jackson (1973)
Soil organic carbon	Rapid titration method	Walkley and Black (1934)
Available nitrogen	Alkaline permanganate method	Subbiah and Asija (1956)
Available phosphorus	0.5 M Sodium bicarbonate using US-VIS spectrophotometer	Olsen et al. (1954)
Available potassium	1 *N* Ammonium acetate using flame photometer technique	Merwin and Peech (1950)
DTPA zinc, copper, iron and managanese	Atomic absorption spectrophotometer	Lindsay and Norwell (1978)

as well as lignin content. The SOC content can be improved due to root anatomy and plant residue through conjoint application through manure and chemical fertilizers (Dhaliwal et al., 2019).

Table 4. Effect of farmyard manure and nitrogen levels on *p*H, EC and SOC of surface (0-15 cm) soil after harvesting of chilli

Treatments		*p*H			EC (dS/m)			SOC (%)		
		FYM		Mean	FYM		Mean	FYM		Mean
		Without	With		Without	With		Without	With	
T_1		7.39	7.28	7.33	0.22	0.18	0.20	0.40	0.42	0.41
T_2		7.35	7.26	7.31	0.20	0.16	0.18	0.41	0.43	0.42
T_3		7.34	7.26	7.30	0.20	0.15	0.18	0.42	0.43	0.42
T_4		7.34	7.25	7.29	0.19	0.15	0.17	0.42	0.44	0.43
T_5		7.33	7.25	7.29	0.19	0.14	0.17	0.41	0.43	0.42
T_6		7.33	7.24	7.29	0.19	0.15	0.17	0.41	0.43	0.42
T_7		7.32	7.23	7.28	0.18	0.15	0.17	0.41	0.43	0.42
T_8		7.31	7.23	7.27	0.18	0.14	0.16	0.42	0.44	0.43
T_9		7.31	7.23	7.27	0.17	0.14	0.15	0.41	0.43	0.42
T_{10}		7.29	7.22	7.26	0.18	0.16	0.17	0.41	0.44	0.42
T_{11}		7.28	7.21	7.24	0.17	0.14	0.15	0.42	0.44	0.43
T_{12}		7.28	7.21	7.25	0.16	0.14	0.15	0.42	0.44	0.43
T_{13}		7.28	7.20	7.24	0.16	0.13	0.15	0.41	0.43	0.42
Mean		7.32	7.24		0.18	0.15		0.41	0.43	
CD (p=0.05)	FYM		0.01			NS			NS	
	Nitrogen		NS			0.01			NS	
	Interaction		NS			NS			NS	

Table 5. Effect of farmyard manure and nitrogen levels on available N, P and K of surface (0-15 cm) soil after harvesting of chilli

Treatments		Available N (kg/ha)			Available P (kg/ha)			Available K (kg/ha)		
		FYM		Mean	FYM		Mean	FYM		Mean
		Without	With		Without	With		Without	With	
T_1		119.8	152.5	136.2	19.6	29.3	24.5	129.8	147.5	138.6
T_2		135.4	161.4	148.4	19.8	29.8	24.8	131.1	149.7	140.4
T_3		137.5	163.4	150.5	20.6	28.5	24.6	131.4	151.5	141.5
T_4		139.8	167.4	153.6	19.3	29.4	24.4	133.6	151.7	142.6
T_5		141.6	172.2	156.9	19.5	28.5	24.0	134.5	154.4	144.4
T_6		144.3	185.5	164.9	20.3	28.7	24.5	132.5	153.3	142.9
T_7		144.4	187.1	165.8	19.4	29.3	24.4	134.5	153.7	144.1
T_8		144.6	191.4	168.0	20.7	29.7	25.2	135.4	156.4	145.9
T_9		145.3	193.5	169.4	19.5	27.7	23.6	132.5	156.9	144.7
T_{10}		147.3	201.6	174.5	19.9	28.4	24.2	133.5	155.3	144.4
T_{11}		148.3	202.2	175.3	19.5	27.9	23.7	133.6	155.5	144.5
T_{12}		149.3	205.1	177.2	19.3	29.5	24.4	133.7	153.3	143.5
T_{13}		152.1	210.2	181.2	20.5	28.2	24.3	133.7	155.5	144.6
Mean		142.3	184.1		19.8	28.8		133.1	153.4	
CD (p=0.05)	FYM		1.06			0.23			0.66	
	Nitrogen		4.89			0.19			NS	
	Interaction		6.70			0.32			NS	

Available primary macronutrients (nitrogen, phosphorus and potassium): The integrated application showed a considerable increase in the available N content of surface soil with respect to initial available N content (137.6 kg/ha). There was a significant difference exhibited without and with FYM on available N content (Table 5). A significantly higher N content (184.1 kg/ha) in FYM plots with respect to without FYM plots (142.3 kg/ha). There was a significant increase in N content of surface (0-15 cm) soil concerning increase in different levels and splits of N-fertilizer and revealed that higher N content (181.2 kg/ha) in T_{13} treatment which was at par with T_{12} treatment. There was significant interaction observed between FYM and N-fertilizer on the available N content of surface soil. The $F_{25}T_{13}$ treatment *i.e.*, 150 kg N/ha was applied in five splits integrated with FYM practices had 210.2 kg N/ha which resulted (42.7%) significantly higher than F_0T_{10} treatment *i.e.*, 150 kg N/ha in two splits without FYM practices. The INM treatments have higher available N content than sole inorganic N-fertilizer treatments due to the improvement of SOC and slow mineralization of available N in surface soil (Guo et al., 2016; Yan et al., 2021). There was a significant difference among without and with treated FYM plots on available P content (Table 5). The FYM treated plots have higher available P (28.8 kg/ha) with respect to without FYM plots (19.8 kg/ha). The T_8 treatment has the highest available P (25.2 kg/ha), which was significantly higher than the rest of all the treatments and the lowest P content (23.6 kg/ha) was observed in T_9 treatment. Significant interaction was observed between FYM and N-fertilizer on the available P content. The $F_{25}T_2$ treatment has significantly higher available P (29.8 kg/ha) than the F_0T_{12} (19.3 kg/ha). The FYM strategies with inorganic N-fertilizer have higher available P content than inorganic N-fertilizer due to soil organic matter (SOM) coating the sesquioxides (Al-P and Fe-P), rendering them inactive and reducing the P fixing capacity of surface soil resulting in improvement of P in soil (Subehia et al., 2013). There was a significant difference among the available K content (Table 5) between the without and with FYM plots and revealed that FYM treated plots resulted higher K content (153.4 kg/ha) than without treated FYM plots plots (133.1 kg/ha). The increase in available K of surface soil with combined application of of N-fertilizer with FYM may be attributed to improves the water holding capacity (WHC) and slow mineralization of nutrients (Meena et al., 2024). The farmyard manure can significantly increase the available K content as compared to inorganic N fertilizer plots (Urkurkar et al., 2010).

Table 6. Effect of farmyard manure and nitrogen levels on DTPA-extractable Zn, Cu, Fe and Mn of surface (0-15 cm) soil after harvesting of chilli

Treatments		DTPA-extractable Zn			DTPA-extractable Cu			DTPA-extractable Fe			DTPA-extractable Mn		
		FYM		Mean	FYM		Mean	FYM		Mean	FYM		Mean
		Without	With		Without	With		Without	With		Without	With	
T_1		2.56	3.17	2.87	0.35	0.52	0.44	11.5	16.4	13.9	3.70	4.78	4.24
T_2		2.58	3.21	2.90	0.37	0.53	0.45	11.8	16.5	14.2	3.81	4.94	4.38
T_3		2.59	3.22	2.90	0.36	0.53	0.45	11.9	16.6	14.3	3.56	4.97	4.26
T_4		2.58	3.24	2.91	0.36	0.54	0.45	11.5	18.2	14.8	4.01	5.10	4.56
T_5		2.60	3.27	2.94	0.38	0.56	0.47	12.1	17.0	14.6	4.13	5.24	4.68
T_6		2.57	3.23	2.90	0.40	0.55	0.48	11.9	17.2	14.6	4.03	5.00	4.52
T_7		2.57	3.24	2.91	0.39	0.54	0.47	12.2	17.5	14.9	4.19	5.12	4.66
T_8		2.59	3.26	2.93	0.37	0.56	0.47	12.4	17.9	15.2	4.08	5.20	4.64
T_9		2.60	3.29	2.95	0.40	0.53	0.46	12.1	18.2	15.1	4.15	5.06	4.61
T_{10}		2.59	3.27	2.93	0.39	0.55	0.47	12.3	18.5	15.4	4.06	5.10	4.58
T_{11}		2.60	3.28	2.94	0.40	0.54	0.47	11.7	17.9	14.8	4.21	5.04	4.62
T_{12}		2.61	3.30	2.95	0.38	0.58	0.48	12.8	18.1	15.4	4.15	5.21	4.68
T_{13}		2.58	3.25	2.91	0.37	0.53	0.45	12.1	17.7	14.9	3.93	5.12	4.53
Mean		2.59	3.25		0.38	0.54		12.0	17.5		4.00	5.06	
CD (p=0.05)	FYM		0.02			0.01			0.08			0.08	
	Nitrogen		NS			NS			NS			NS	
	Interaction		NS			NS			NS			NS	

DTPA-extractable micronutrients (Zn, Cu, Fe and Mn): The integrated application of chemical N-fertilizer with FYM showed a considerable increase in micronutrients *i.e.*, Zn, Cu, Fe and Mn of surface soil in comparison with initial soil samples. There was significantly higher available micronutrients in FYM treated plots than without FYM plots (Table 6). There was non-significant increase in an available micronutrients of soil with respect to increase in different levels and splits of N-fertilizer. The lowest micronutrients were observed in the T_1 (no application of N fertilizer). The INM treatments have higher available micronutrients than sole inorganic N fertilizer due to increasing soil microbial activity during mineralization and decomposition of organic matter which improved the DTPA-extractable Zn, Cu, Fe and Mn content in surface soil by preventing leaching, fixation and precipitation (Walia et al., 2010). Integrated management plots have higher micronutrients as compared to inorganic N-fertilizer due to increasing microbial activity during mineralization and decomposition of SOM (Dhaliwal et al., 2020).

Fruit yield (q/ha): The higher fruit yield (229.1 q/ha) was obtained in FYM treated plots than without FYM treated plots (169.8 q/ha) (Table 7). The T_8 treatment *i.e* 113 kg N/ha application in four equal splits produced significantly higher fruit yield (254.4 q/ha). There was significant interaction observed between FYM and N-fertilizer on fruit yield and showed that $F_{25}T_8$ treatment resulted 25.6 % significantly higher fruit yield than F_0T_{12} treatment. With in each N-fertilizer level *i.e.*, 75, 113 and 150 kg/ha, four split doses performed best followed by three, two and five split doses. The fruit yield is the total quantity of mature red ripe fruits produced per unit of land. It is influenced by many parameters, besides its most important parameter for attaining monetary returns for farmers. Nitrogen is an important deficient plant nutrient that affect the fruit productivity of chilli (Khan et al., 2014).

Table 7. Effect of farmyard manure and nitrogen levels on total fruit yield (q/ha) of chilli

Treatments		Red ripe fruit yield		
		FYM		Mean
		Without	With	
T_1		104.7	188.0	146.3
T_2		140.2	207.6	173.9
T_3		158.0	220.8	189.4
T_4		180.4	234.5	207.5
T_5		124.9	196.8	160.9
T_6		171.6	248.2	209.9
T_7		184.4	269.9	227.1
T_8		206.2	302.6	254.4
T_9		143.8	217.2	180.5
T_{10}		196.6	219.3	208.0
T_{11}		208.7	237.6	223.2
T_{12}		225.2	245.9	235.5
T_{13}		162.8	189.7	176.3
Mean		169.8	229.1	
CD (p=0.05)	FYM		4.30	
	Nitrogen		7.40	
	Interaction		10.7	

CONCLUSIONS

The integrated application of farmyard manure and nitrogen fertilizer practices resulted optimal fruit yield along with builds-up of macro and micronutrients for sustainable growth and development of chilli. The higher fruit yield and nutrients availability was attained with 113 kg N/ha applied in four equal splits. There was significantly higher fruit yield by substitution of 113 kg N/ha applied in four equal splits than RDF treatment *i.e.*, 75 kg N/ha applied in two split doses. Within each N level: 75, 113 and 150 kg N/ha, four equal splits of N-fertilizer performed better than three, two and five splits, respectively both in without and with FYM plots. Only two splits of N-fertilizer were not ensured the optimal fruit yield and nutrients availability as per plant demands. Therefore, 113 kg N/ha applied in four equal splits can be recommended to farmers for achieving higher fruit yield and agricultural sustainability.

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AUTHOR'S CONTRIBUTION

Conceptualization and designing of research work (DK,AG); Execution of field/lab experiments and data collection (DK); Analysis of data and interpretation (DK, AG, VS); Preparation of manuscript (DK).

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Boron Seed Priming Impacts on Germination Percentage and Seed Boron Content under Controlled Conditions

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Abstract: Okra, a crop with high boron (B) demand, faces low germination due to hard seed coat, limiting yield potential. This study evaluated the impact of B seed priming on the germination percentage and seed B content of okra under controlled conditions. Seeds were primed with graded B concentrations (1.0%, 0.5%, 0.1%, 0.05%, and 0.01%) and hydroprimed (distilled water) for three durations (12, 24, and 36 hours). B seed priming significantly enhanced germination and seed B content, except at higher B concentrations, where toxicity inhibited germination. The highest germination percentage (96.7%) was achieved with 0.05% B for 24 hours, aligning with the calculated optimum concentration of 0.048% B. Longer durations (36 hours) resulted in higher B content (up to 787.9 mg kg^{-1} with 0.1% B), germination percentage declined due to toxicity. These findings highlight the potential of B seed priming at optimal concentrations to address B deficiencies and improve okra germination, particularly in B-deficient soils.

Keywords: Germination percentage, Nutrient seed priming, Seed B content

Okra {*Abelmoschus esculentus* (L.) Moench} crop thrives well in tropical, subtropical, and warm temperate regions (Singh et al., 2014). The crop recognized for its nutritional value and culinary purpose has annual production of around 9.96 million tons (FAOSTAT 2020). However, its hard seed coat leads to low germination, hindering the crop from reaching its full yield potential. Seed priming is an cost-effective method for even uniform germination, fast emergence, and improved seedling vigour (Mondal and Bose 2019) leading to enhanced yields under stressed environments (Singh et al., 2015). Nutrient priming is a distinctive technique employed under nutrient-stressed conditions to improve germination rates and provide an initial boost to crops facing specific nutrient deficiencies.

Globally, major nutrients are commonly applied for crop production in fertilizer form, while the use of other nutrients is often overlooked, leading to widespread multi-nutrient deficiencies. Among micronutrients, B is the most deficient nutrient not only in India but throughout the world. B plays indispensable role in plants for translocation of sugars, synthesis of nucleic acid, carbohydrate and nutrient metabolism, cell division and disease resistance (Shireen et al., 2018). Furthermore, B stimulates seed germination and seedling emergence (Iqbal et al., 2017), suggesting that nutrient priming with B in okra seeds could address the issue of low germination. To evaluate this premise, an experiment was conducted to assess the impact of graded doses and durations of B seed priming on okra germination and nutrient content.

MATERIAL AND METHODS

Seed priming: Experiment was conducted on okra *var.* Palam Komal in the laboratory of Soil Science Department of CSKHPKV, Palampur during 2021. Initially the moisture content was determined by grinding the seeds, drying at 130°C for 4 hours and was 9.8% on a fresh weight basis. For priming, the okra seeds were soaked in boric acid concentrations (1.0, 0.5, 0.1, 0.05, and 0.01%), and hydro-priming (distilled water) for 12, 24 and 36 hours duration, respectively. The hydro-priming treatment, containing 0% boric acid, served as the control. After completion of priming, the treated seeds were washed with distilled water and air-dried back to their original moisture content.

Germination and seed B content: Ten seeds were arranged in each petri plate on moist seed germination paper arranged according to completely randomized design with three replications. Water was uniformly applied using a pipette to avoid non uniformity among the treatments in terms of moisture. The seeds were incubated at 25 ± 2°C up to 8 days. Seed was considered germinated when the radical pierced the coats up to 2 mm.

Okra seeds with and without priming were analyzed for their B content taking 1g oven dried (at 60°C) and crushed seeds using Azomethine-H method as outlined by Datta et al. (1998).

Statistical analysis: The laboratory data were analyzed statistically using analysis of variance (ANOVA) for a factorial completely randomized design.

RESULTS AND DISCUSSION

Germination percentage: Seed priming with different B concentrations significantly impacted the germination percentage, that varied between 8.9 and 90%. Among the treatments, 0.05% B priming exhibited the highest germination percentage, significantly outperforming all other treatments. This was followed by 0.01% B, which also showed a significant improvement over the hydropriming treatments. Hydropriming resulted in moderate germination, statistically comparable to 0.1% B, but significantly lower than 0.05% B and 0.01% B. Conversely, higher concentrations of B (0.5% and 1.0%) led to a significant decline in germination, with 1.0% B showing the lowest. No significant differences were observed among the overall means, indicating that the duration of priming did not significantly influence germination percentage when averaged across all concentrations.

The metabolic processes involved in early phases of germination are stimulated by seed priming, resulting in faster and more uniform germination in B-primed seeds (Iqbal et al., 2017). The role of seed priming in enhancing germination percentages has been well-documented in various crops. Studies by Keshavarz et al. (2011), Noor-un-Nisa et al. (2013), and Geetha et al. (2018) provide evidence of the effectiveness of seed priming in improving germination and early seedling vigour, supporting the findings of the present study. Furthermore, low germination percentages at higher levels of B seed priming may be attributed to its toxicity, arising from the narrow range between optimal and toxic levels of B.

Seed B content: The significant variation in the seed B content at varying levels was observed, ranging between 38.5 and 643.4 mg kg^{-1} (Table 2) with different priming concentrations and durations. Hydropriming (0% B) resulted in the lowest seed B content across all durations. Among the B treatments, 0.1% B consistently produced the highest seed B content, significantly outperforming all other concentrations. This was particularly evident at the 36-hour duration, which showed the highest B content overall. At 0.05% B, seed B content was significantly higher than 0.01% B and hydropriming, with 36-hour duration, yielding better results compared to shorter durations. However, at higher concentrations (0.5% and 1.0% B), B content decreased due to potential toxicity, although it remained significantly higher than hydropriming. When comparing durations, the 36-hour priming consistently led to the highest seed B content across most treatments, followed by 24 and 12 hours. The increase in seed B content resulted in increased germination of soybean (Cirak et al., 2006). However, the positive relationship between seed B content and germination existed

Table 1. Effect of priming concentrations and durations on the germination percentage

Treatments	Priming duration (hour)			Mean
	12	24	36	
0 % B (Hydropriming)	70.0^{f}	76.7^{ef}	83.3^{cde}	76.7^{C}
0.01 % B	76.7^{ef}	86.7^{bcd}	90.0^{abc}	84.4^{B}
0.05 % B	80.0^{de}	96.7^{a}	93.3^{ab}	90.0^{A}
0.1 % B	70.0^{f}	80.0^{de}	76.7^{ef}	75.6^{C}
0.5 % B	30.0^{g}	20.0^{h}	0.0^{i}	16.7^{D}
1.0 % B	20.0^{h}	6.7^{i}	0.0^{i}	8.9^{E}
Mean	57.8^{ns}	61.1^{ns}	57.2^{ns}	

Note: Values followed by different lowercase letters across durations indicate significant differences, while different uppercase letters represent significant differences in the respective means. "ns" denotes non-significant differences within the means

Table 2. Effect of priming concentrations and durations on seed B content (mg kg^{-1})

Treatments	Priming duration (hour)			Mean
	12	24	36	
0 % B (Hydropriming)	39.3^{l}	40.0^{l}	40.5^{l}	39.9^{F}
0.01 % B	120.5^{k}	142.5^{k}	227.0^{j}	163.3^{E}
0.05 % B	349.5^{i}	493.9^{e}	652.7^{b}	498.7^{B}
0.1 % B	530.9^{d}	611.4^{c}	787.9^{a}	643.4^{A}
0.5 % B	514.0^{e}	466.8^{f}	407.9^{h}	462.9^{C}
1.0 % B	494.5^{e}	436.6^{g}	353.8^{i}	428.3^{D}
Mean	341.4^{C}	365.2^{B}	411.6^{A}	

Note: Values followed by different lowercase letters across durations indicate significant differences, while different uppercase letters represent significant differences in the respective means

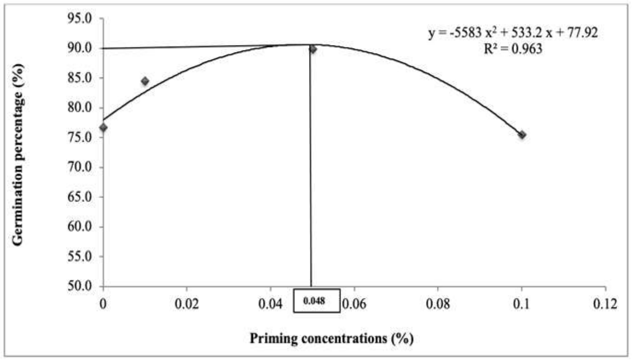

Fig. 1. Optimum priming concentration under controlled conditions

up to a certain B level, emphasising an optimal B content for priming the seeds. Contrarily, the evident decline in seed germination at high B priming concentration may be due to higher accumulation in the seed leading to toxicity. Khan et al. (2006) suggested that very little quantity of B is needed to regulate the meristematic growth, therefore, excessive B becomes toxic which impedes the normal growth (Bonilla et al., 2004). High B concentration was reported to decrease the germination percentage and germination rate of wheat (Ashagre et al.,2014). Thus, underlining the necessity of an optimum seed priming concentration for B due to a narrow range of deficiency and toxicity.

Optimum B priming concentration under laboratory conditions: The quadratic curve was obtained with R^2 value of 0.963 which was higher compared to the linear model. The equation generated was subjected to the first derivative and then equated to zero (Fig. 1). The optimum concentration of B required for attaining the maximum germination of okra was B @ 0.048% under controlled conditions

CONCLUSIONS

B priming at optimal concentrations significantly enhanced seed germination and B content, with the highest germination percentage observed at 0.05% B for 24 hours. However, higher concentrations led to toxicity, underscoring the narrow margin between deficiency and toxicity. The optimal concentration for maximum germination was determined to be 0.048% B. Additionally, longer priming durations (36 hours) resulted in higher seed B content, highlighting the efficacy of B priming in addressing B deficiencies in okra. Future field studies are essential to validate these findings and explore their practical applicability in diverse agro-ecological conditions.

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Popularizing Millet Cultivation: Optimising Crop Spacing and Identifying A Suitable Variety of Pearl Millet (*Pennisetum glaucum*) for Southern Laterites of Kerala

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Abstract: Millets are gaining popularity in the changing scenario of global climate, based on their ability to withstand harsh weather conditions, adaptability in marginal lands and also because of their nutritional significance. Standardisation of the agro techniques of pearl millet could lead to popularisation of the crop and experiment was conducted at College of Agriculture, Vellayani, KAU in identifying a suitable variety and optimum spacing for pearl millet grown in Southern laterites of Kerala. Five pearl millet varieties *viz*., ICMV 221, VPMV 9, MH 946, ICTP 8203 and CO 10 were experimented under two spacings i.e., 30 cm X 20 cm and 45 cm X 15 cm during Rabi, 2022 and Summer, 2023. Among varieties, CO 10 performed significantly better in terms of growth attributes, uptake of major nutrients and yield performance followed by ICMV 221.The crop spacing, 30 cm X 20 cm exhibited significantly higher growth, nutrient uptake, and yield in comparison to 45 cm X 15 cm. The interaction effect of varieties and spacing was also significant with CO 10 performing significantly better growth, nutrient uptake and yield under 30 cm x 20 cm spacing. The study recommends cultivation of CO 10 in the southern laterites of Kerala under a crop spacing of 30 cm x 20 cm, optimum for growth and yield.

Keywords: Pearl millet, Varieties, Spacing, Growth, Nutrient uptake, Yield

Global climate change and extreme weather fluctuations have emerged the most threatening challenges to agriculture particularly in the production of important cereals crops. In this scenario, millets have emerged climate smart crops for next generation agriculture. Millets have the ability to withstand high temperatures, drought and poor soil conditions which makes them invaluable assets in sustainable agriculture. Millets are rich sources of dietary fibres, vitamins, and minerals (Chaurasia and Anichari 2023). Due to their exceptional nutritional profile and potential health benefits, millets are also regarded as “nutraceuticals” as well as 'famine reserves' considering their prolonged shelf life.

Pearl millet (*Pennisetum glaucum* L.) is an important millet crop having multipurpose utility as food feed and forage. The low glycaemic index and gluten free nature of the grain makes it a preferred crop for those having gluten allergies and diabetes. In India, pearl millet production was approximately 17.32 million tonnes during 2022-2023 with a productivity of 1401 kg per hectare (India data insights 2023). Pearl millet is being popularized as a market-oriented crop owing to the varied uses such as animal feed, potable alcohol, processed food etc (Yadav and Rai 2013).

Optimum spacing allows plants to receive adequate sunlight, nutrients and water, which are essential for better growth and establishment. It also helps to prevent overcrowding, thereby eliminating any competition for soil water and nutrients. The soil and climatic conditions of Kerala are congenial for growing a variety of millets as experienced from success stories of several farmers. However, as the agrotechniques for most of the millets are not standardised for Kerala conditions, this experiment aimed in promoting cultivation of a major millet crop *i.e*., Pearl millet in the southern laterites (Agro Ecological Unit - 8) of Kerala by way of identifying a suitable variety for the region and also optimising the crop spacing for better crop yield.

MATERIAL AND METHODS

The study was conducted during *Rabi*, 2022 (September 2022 - January 2023) and summer, 2023 (March to July 2023) at College of Agriculture, Vellayani. The experimental field was located at 8°25'39" N latitude and 76°59' 9" E longitude, at an altitude of 19 m above msl. The soil was sandy loam belonging to the order Oxisol with acidic pH (5.6), safe electrical conductivity (0.2 ds m^{-1}) and medium organic carbon content (1.23 %). The soil was low in available nitrogen (247.31 kg ha^{-1}), high in available phosphorus (247.31 kg ha^{-1}) and medium in available potassium (226.5 kg ha^{-1}).

Pearl millet varieties ICMV 221, VPMV 9, MH 946, ICTP 8203 and CO 10 were cultivated under two different crop spacings of 30 cm X 20 cm and 45 cm X 15 cm. The crop was maintained as per the package of practices recommendations of Kerala Agricultural University (KAU 2016). Seeds at the rate of 4 kg ha^{-1} were dibbled. Farmyard manure was applied at the rate of 5 t ha^{-1} at the time of land preparation. N, P and K fertilizers were applied at the rate of 70:35:35 kg ha^{-1} for crop nourishment. Entire dose of phosphorus and potash was applied as basal. Nitrogen was applied at two equal splits, half as basal and the rest at 30 DAS.

Biometric observations were periodically recorded and the grain and straw yield from respective treatment plots were recorded at harvest. Nitrogen content of plants was analysed using the modified microkjeldahl method (Jackson 1973). Phosphorus content was analysed using Vanadomolybdate phosphoric yellow colour method (Piper 1966). Potassium content was analyzed using flame photometer method. The plant nutrient contents were then multiplied with total dry matter production to obtain the uptake of respective nutrients and expressed in kg ha^{-1} (Piper 1966). The data generated from the experiment were statistically analyzed using analysis of variance technique (Gopinath et al., 2021).

RESULTS AND DISCUSSION

Growth parameters: At all the stages i.e., 40, 60 DAS and at harvest, significant enhancement in plant height was observed with variety CO 10 followed by ICMV 221 (Table 1). Similar trend was observed for DMP which was significantly higher in CO 10 followed by ICMV 221. Probably, the superior genetic traits of CO 10 varieties contributed to enhanced growth (Sumathi et al., 2017). The uptake of major

Table 1. Varietal differences and crop spacing in influencing the growth attributes of pearl millet

Treatments	Plant height (cm)						DMP (g)					
	Rabi 2022			Summer 2023			*Rabi* 2022			Summer 2023		
	40 DAS	60 DAS	Harvest	40 DAS	60 DAS	Harvest	40 DAS	60 DAS	Harvest	40 DAS	60 DAS	Harvest
Variety (v)												
v_1: ICMV221	100.08	166.00	186.25	87.58	160.08	182.00	32.22	57.47	94.95	29.62	54. 11	91.20
v_2: VPMV9	95.25	164.00	181.58	82.75	156.60	177.42	29.62	53.61	93.30	27.40	51.91	89.63
v_3: MH946	87.60	162.08	174.83	74.10	153.73	170.58	28.40	50.47	90.09	26.33	47.00	86.70
v_4: ICTP8203	94.08	164.75	185.42	81.58	159.08	182.08	30.71	54.97	94.65	28.84	53.23	90.98
v_5: Co-10	104.21	172.33	189.42	93.46	168.08	185.92	33.48	59.05	96.95	30.66	56.05	92.64
CD (p=0.05)	4.631	3.324	3.141	4.631	3.794	3.179	1.038	1.079	0.995	0.989	1.61	1.745
Spacing (s)												
s_1: 30X 20cm	101.67	167.93	188.23	91.27	162.53	185.33	32.175	56.80	96.22	53.42	92.47	53.42
s_2: 45X 15cm	90.82	163.73	178.77	76.52	156.50	173.87	29.598	53.44	91.76	51.50	87.98	51.50
CD (p=0.05)	2.929	2.102	1.987	2.929	2.399	2.011	0.656	0.682	0.629	1.018	1.104	1.018
Interaction (v X s)	Plant height (cm)						DMP (g)					
	Rabi 2022			Summer 2023			*Rabi* 2022			Summer 2023		
	40 DAS	60 DAS	Harvest	40 DAS	60 DAS	Harvest	40 DAS	60 DAS	Harvest	40 DAS	60 DAS	Harvest
v_1s_1	104.17	167.00	190.33	94.17	162.17	186.83	33.76	58.81	96.60	29.86	55.14	93.45
v_1s_2	96.00	165.00	182.17	81.00	158.00	177.17	30.68	56.14	93.30	29.38	53.07	88.96
v_2s_1	97.33	163.67	186.17	87.33	156.50	182.67	30.17	54.75	95.67	27.02	51.13	91.68
v_2s_2	93.17	164.33	177.00	78.17	156.70	172.17	29.08	52.47	90.94	27.78	52.70	87.59
v_3s_1	96.33	166.50	183.67	84.33	159.67	180.17	29.89	53.08	93.30	27.96	49.93	90.73
v_3s_2	78.87	157.67	166.00	63.87	147.80	161.00	26.91	47.88	86.89	24.69	44.07	82.66
v_4s_1	98.17	166.50	187.83	88.17	162.50	185.83	31.15	56.24	96.46	28.64	53.26	92.22
v_4s_2	90.00	163.00	183.00	75.00	155.67	178.33	30.27	53.70	92.83	29.05	53.21	89.73
v_5s_1	112.33	176.00	193.17	102.33	171.83	191.17	35.90	61.11	99.07	31.48	57.64	94.30
v_5s_2	96.08	168.67	185.67	84.58	164.33	180.67	31.06	57.00	94.83	29.84	54.46	90.98
CD (p=0.05)	6.550	4.700	4.442	NS	5.365	4.496	1.468	1.526	1.407	1.398	2.276	2.468

nutrients N, P and K at harvest stage was significantly higher with varieties V_5 and V_1 (Table 3) which could be related with the enhanced growth attributes for these varieties at harvest.

Under the spacing S_1 (30 cm X 20 cm), significantly taller plants and enhanced DMP were observed compared to S_2 at all the growth stages under study. Among V x S interactions, V_5S_1 (CO 10 under 30 cm X 20 cm) recorded significant enhancement with respect to plant height and DMP at all the growth stages under study. Plants grown close can shade each other which can reduce the amount of sunlight they receive. Mutual competition for sunlight can therefore be severe under closer plant to plant spacing (Singh et al., 2012). Under $S_{1,}$ plant to plant spacing of 20 cm was maintained whereas in $S_{2,}$ was 15 cm. As plant to plant spacing increases, due to less number of plants within the row, there is increased availability of resources including sunlight, moisture and nutrients for individual plants, which promotes more vigorous growth and development (Legese 2024).

Dry weight is a net result of plant height, number of tillers and leaf area. Spacing S_1 (30 cm X 20 cm) had significantly more number of tillers (Table 4) which could be related with the increased DMP under S_1. The plant population under S_1 was 1.66,667 plants per hectare whereas under $S_{2,}$ it was 1,48,148 plants. Higher plant population results in increased leaf area index due to more green canopy cover per unit area. This in turn results in enhanced photosynthesis and hence more accumulation of photosynthates contributing to higher

Table 2. Varietal differences and crop spacing in influencing the nutrient uptake of pearl millet

Treatments	NPK uptake (kg ha^{-1})					
	Rabi 2022			Summer 2023		
	N uptake (kg ha^{-1})	P uptake (kg ha^{-1})	K uptake (kg ha^{-1})	N uptake (kg ha^{-1})	P uptake (kg ha^{-1})	K uptake (kg ha^{-1})
Variety (v)						
v_1: ICMV221	91.28	19.25	93.29	83.87	13.24	80.56
v_2: VPMV9	80.28	17.77	89.64	78.39	11.94	74.23
v_3: MH946	74.58	13.76	84.45	73.23	9.35	69.09
v_4: ICTP8203	84.86	18.74	91.39	81.24	12.35	76.71
v_5: Co-10	95.09	21.97	96.59	86.64	14.54	83.86
CD (p=0.05)	3.405	1.155	2.985	3.984	1.266	3.302
Spacing (s)						
s_1: 30X 20cm	90.62	20.57	92.51	85.09	13.89	78.51
s_2: 45X 15cm	79.82	16.03	89.64	76.25	10.67	75.27
CD (p=0.05)	2.153	0.73	1.888	2.520	0.801	2.089
Interaction (v X s)	NPK uptake (kg ha^{-1})					
	Rabi 2022			Summer 2023		
	N uptake (kg ha^{-1})	P uptake (kg ha^{-1})	K uptake (kg ha^{-1})	N uptake (kg ha^{-1})	P uptake (kg ha^{-1})	K uptake (kg ha^{-1})
v_1s_1	94.65	20.82	93.76	90.19	15.06	83.09
v_1s_2	87.90	17.68	92.82	77.54	11.42	78.04
v_2s_1	86.36	19.44	89.36	79.46	12.59	73.12
v_2s_2	74.20	16.09	89.92	77.32	11.28	75.33
v_3s_1	83.09	17.16	88.79	76.43	10.78	71.73
v_3s_2	66.08	10.36	80.12	70.03	7.913	66.46
v_4s_1	89.78	20.71	92.05	85.05	13.35	77.16
v_4s_2	79.94	16.77	90.73	77.42	11.35	76.27
v_5s_1	99.22	24.69	98.59	94.34	17.67	87.46
v_5s_2	90.96	19.26	94.59	78.94	11.40	80.27
CD (p=0.05)	4.815	1.633	4.221	5.635	1.790	4.670

DMP (Sali 2022). Availability of sunlight is yet another major factor for better photosynthesis. Under wider plant to plant spacing, individual plants receive more sunlight resulting in better photosynthesis contributing to more dry matter accumulation (Qodliyati et al., 2018). Virat and Singh (2021) also identified that narrow row spacing resulted in increased dry matter accumulation in pearl millet attributed to efficient photosynthesis. Rana et al. (2013) observed that varieties influenced the total dry matter accumulation. Growth enhancement was with CO 10 variety and spacing of 30 cm X 20 cm reflected in significantly enhanced performance under interactions (V_5S_1) as well. Under a wider intra row spacing of 20 cm, probably proper utilization of space and sunlight occurred and there was a reduction in competition between plants for these resources which lead to improved growth.

Nutrient uptake: Results on the effect of different varieties, spacing and their interaction on nutrient uptake of pearl millet are presented in. Significant enhancement in N, P and K uptake was observed with variety CO 10 followed by ICMV 221 (Table 3). S_1 (30 cm X 20 cm) recorded significantly higher uptake of N, P and K. Among the interactions, V_5S_1 resulted in significantly highest N,P and K uptake (N-99.22 kg ha^{-1}, P-24.69 kg ha^{-1}, K-98.59 kg ha^{-1} during *Rabi* and N-94.34 kg ha^{-1}, P-17.67 kg ha^{-1}, K-87.46 kg ha^{-1} during summer). DMP was significantly higher with CO 10, closely followed by ICMV 221 (Table 1). As nutrient uptake is greatly influenced by dry matter accumulation, significantly higher uptake of nutrients under V_5 and S_1 could be well explained on the basis of higher DMP. Under S_1 (30 cm x 20 cm), the DMP was significantly higher compared to S_2 the plant to plant spacing being more in S_1,

Table 3. Varietal differences and crop spacing in influencing the yield and ear head weight of pearl millet

Treatments	NPK uptake (kg ha^{-1})					
	Rabi 2022			Summer 2023		
	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Ear head weight (g)	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Ear head weight (g)
Variety (V)						
v_1: ICMV221	2323.96	6216.63	33.62	2148.51	6051.33	32.22
v_2: VPMV9	2208.33	5805.12	31.40	1775.99	5756.01	29.62
v_3: MH946	1992.29	5691.47	30.33	1614.51	5196.38	28.40
v_4: ICTP8203	2264.58	6040.13	32.84	1878.77	5910.90	30.71
v_5: Co-10	2469.37	6664.35	34.66	2385.10	6507.98	33.48
CD(p=0.05)	67.68	206.261	0.989	240.662	258.456	1.038
Spacing (s)						
s_1: 30X 20cm	2314.19	6342.82	32.99	2164.77	6161.38	32.175
s_2: 45X 15cm	2189.23	5824.26	32.15	1756.38	5607.69	29.598
CD (p=0.05)	42.805	130.451	0.625	152.208	163.462	0.656
Interaction (v X s)	NPK uptake (kg ha^{-1})					
	Rabi 2022			Summer 2023		
	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Ear head weight (g)	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Ear head weight (g)
v_1s_1	2318.75	6347.92	33.86	2148.48	6168.96	33.76
v_1s_2	2329.17	6085.35	33.38	2148.55	5933.70	30.68
v_2s_1	2247.91	6104.17	31.02	2072.22	5953.70	30.17
v_2s_2	2168.75	5506.08	31.78	1479.76	5558.49	29.08
v_3s_1	2157.28	6012.50	31.96	2012.64	5742.30	29.89
v_3s_2	1827.30	5370.45	28.69	1216.39	4650.46	26.91
v_4s_1	2281.25	6179.17	32.64	2095.40	6016.68	31.15
v_4s_2	2247.92	5901.10	33.05	1662.14	5805.11	30.27
v_5s_1	2565.73	7070.37	35.48	2495.11	6925.26	35.90
v_5s_2	2373.00	6258.33	33.84	2275.09	6090.70	31.06
CD (p=0.05)	95.714	291.697	1.398	340.348	365.512	1.468

Table 4. Varietal differences and crop spacing ontiller numbers and productive tillers at harvest stage

Treatments	Number of tillers		Number of productive tillers	
	Rabi 2022	Summer 2023	*Rabi* 2022	Summer 2023
Variety (v)				
v_1: ICMV221	40.17	36.33	25.30	22.78
v_2: VPMV9	30.17	31.67	20.98	21.27
v_3: MH946	26.83	29.00	17.48	19.09
v_4: ICTP8203	38.60	32.67	23.55	22.55
v_5: Co-10	42.67	40.00	28.80	25.00
CD (p=0.05)	3.652	1.863	4.693	1.684
Spacing (s)				
s_1: 30X 20cm	36.87	36.67	24.06	22.32
s_2: 45X 15cm	34.53	31.20	22.38	21.96
CD (p=0.05)	2.309	1.178	NS	NS
Interaction (v X s)	Number of tillers		Number of productive tillers	
	Rabi 2022	Summer 2023	*Rabi* 2022	Summer 2023
v_1s_1	41.33	38.67	25.92	22.44
v_1s_2	39.00	34.00	24.68	23.11
v_2s_1	31.00	35.00	22.21	21.96
v_2s_2	29.33	28.33	19.75	20.59
v_3s_1	29.00	33.00	18.51	19.29
v_3s_2	24.67	25.00	16.46	18.89
v_4s_1	41.00	35.33	24.06	22.22
v_4s_2	36.33	30.00	23.04	22.89
v_5s_1	42.00	41.33	29.62	25.67
v_5s_2	43.33	38.67	27.97	24.33
CD (p=0.05)	NS	NS	NS	NS

individual plant got access to more space and other resources including sunlight contributing to better growth parameters viz, plant height (Table 1), number of tillers (Table 4). As DMP and nutrient uptake are closely related, high nutrient uptake observed with S_1 could be attributed to higher DMP.

Yield parameters and yield : Variety V_5 recorded the highest grain and straw yield for rabi and summer crops closely followed by ICMV 221(Table 2). The spacing, S_1 (30 cm X 20 cm) recorded highest grain and straw yield during both the seasons. Among V x S interactions, V_5S_1 recorded significant enhancement with respect to grain yield and straw yield. Significant enhancement in ear head weight of pearl millet was with the variety V_5 (34.66 g during *Rabi* and 33.48 g during summer) which can be related with the higher yield. V_1 was closely followed V_5 in yield performance. The ear head lengths of varieties V_5 and V_1 were higher and comparable and can be related to their better yield performance. Interaction V_5S_1 exhibited significantly higher ear head weight (35.48 g during *Rabi* and 35.90 g during summer) which can be considered as a cumulative effect of the improved individual performance of the treatments. Sumathi et al., (2017) observed CO 10 exhibited better yield performance attributed to its superior genetic traits at Tamil Nadu Agricultural University, Coimbatore. With regard to crop spacing, the better performance of S1 could be well explained on the basis of improved DMP and resultant higher nutrient uptake as detailed above. Srivastava et al. (2015) also observed higher NPK uptake leading to higher yield.

CONCLUSION

The present study identified CO 10 as the best performing variety in terms of growth and yield under the soil and climatic conditions of southern laterites of Kerala. Crop spacing of 30 cm x 20 cm was found superior with regard to growth and yield. interaction effect of CO 10 (V_5) and spacing 30 cm x 20 cm was found promising and hence this treatment combination could be recommended among millet farmers for better prospects in the southern laterites of Kerala.

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Effect of Ascorbic Acid and Harvest Date on Various Traits and Yield Loss of Corn

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Abstract: Experiment was conducted to study the effect of ascorbic acid and harvest date on various traits and yield of corn, an at the College of Agriculture Research Station, Tikrit University. The experiment consisted of three local varieties of corn (Baghdad, Sara, and Fajir), three concentrations of ascorbic acid (0, 100, and 200 mg L^{-1}), and three harvesting date (30, 40, and 50 days) after pollination. There were no significant differences among varieties for studied traits. The ascorbic acid 200 mg L^{-1} resulted in significantly higher broken grains percentage and broken grain (2.587% and 180.09 kg h^{-1}, respectively). Furthermore, this treatment produced higher net grain yield of 6790 kg h^{-1}. Harvest date had significant effect on all studied traits and moisture percentage was decreased significantly by 15% between the first and third dates of harvest and remaining grains percentage in cob after shelled and its weight were increased. Percentage of broken grains was increased from 0.771 to 3.563%, weight increased from 54.23 to 232.94 kg h^{-1} and total grain loss increased from 1.301 to 6.972% as harvesting early from 50 to 30 days after pollination, while the net grain yield was decreased from 7203 to 6080 kg h^{-1} for the same two dates, respectively.

Keywords: Corn, *Zea mays,* Ascorbic acid, Percentage loss

Corn (*Zea mays* L.) is an important crop in Iraq and is cultivated during spring and fall seasons. In Iraq, the importance of this crop is the direct use as human nutrition and corn grain is used to feed animals, poultry and livestock, due to its high nutritional value. Although the importance of this crop, the cultivated areas were decreased to 76 thousand hectares in 2016 with a productivity rate of 3.4 tons h^{-1} (FAO 2018). The most important reasons for the decline in the cultivation and low productivity/unit area of this crop is the presence of gypsum soils in Iraq in more than 8 million hectares. Gypsum soils are characterized by their weakness in terms of fertility, chemical and physical characteristics, in addition to the problems of planting and harvesting dates during spring and fall seasons, and irrigation and water provision problems (Khairo 2016). The important problem facing this crop is the date of harvest and high moisture content in the grain and yield loss, whether by mechanical or manual harvesting, cleaning and shelling of grains. In addition, locally developed genotypes suitable for cultivation in gypsum soil and harvest dates without affecting the yield are important matters for improving the growth and yield of this crop and reducing the loss of the yield.

In order to overcome these problems, it is useful to use some growth stimulants such as plant growth regulators and vitamins to improve the yield, including ascorbic acid, which play an important role in increasing cell division and elongation, improving shoot and root growth, and activating carbon representation as an electron donor (Abrahamian and Kantharajan 2011). Furthermore, in order to reduce the damage of gypsum soil, the identification of the appropriate harvest date is a great importance to minimize damage of the yield and reduce its loss rates. Timely harvest of crop is important when moisture in the grain drops below 20%. Early harvest leads to high moisture content in the grain, but reduces the time the crop stays in the field to exploit the land for the subsequent crop. On the other hand, it causes yield loss during harvest, shelling, and cleaning when the harvest is delayed for a long period (Metz 2006), and high moisture content in grain at harvest increases the loss to 11.73% when mechanically harvested. Therefore, the research aims to test the effect of three concentrates of ascorbic acid on losses in the grain yield at three harvest date.

MATERIAL AND METHODS

Experiment was conducted at College of Agriculture, Tikrit University in gypsum soil (17% gypsum) during 2017 fall season. The experiment was carried out with a randomized complete block design with a split-split plot arrangement of treatments in three replications. The treatments were three local varieties of corn (Baghdad, Sara, and Fajir), three concentrations of ascorbic acid (0, 100, and 200 mg L^{-1}), and three harvest date (30, 40, and 50 days) after pollination. Varieties were allocated to the main plots; ascorbic acid concentrations were allocated to sub plots, harvest date to sub-sub plots. Planting date was on July 15, 2017 with six rows for each treatment with distance of 75 cm and 25 cm in rows and among plants. Phosphate fertilizer (super calcium phosphate), 200 kg.h^{-1}, was added with the first batch of nitrogen fertilizer (Urea 46% N) at the planting date. The second and third batches were added at six leaves stage and inflorescences stage, respectively at 400 kg h^{-1}. Plants were sprayed with ascorbic acid at four leaves stage with a diffuser material to reduce the surface tension and to facilitate the penetration and spread of the solution into the leave tissues. When the plants reached the stage of emergence male and female inflorescences, paper bags were placed on them, then plants were manually pollinated and the pollination dates were recorded. After that, bags were re-placed on the ears for two weeks, then they were removed and the harvested at deferent days (20, 30, 40, and 50 days after the recorded pollination dates). Ears were harvested according to the type of treatment of the harvest date with 10 plants for each treatment taken randomly at the same day.

Sample of seeds was taken from each harvested ear representing one row and weighed immediately after harvesting, and was dried in an electric oven at 70° C until constant Moisture content of the grains was calculated by using the following equation:

$$\%\text{ remaining grains in cob} = \frac{\text{Number of remaining grain in cob}}{\text{Total number of grain}} \times 100$$

Ears were naturally dried to a moisture of 14%, and then were shelled by shelling machine, and the following studies were taken:

Percent remaining grains in the cob after shelling:

$$\%\text{ broken grain} = \frac{\text{Number of broken grain}}{\text{Total number of grain}} \times 100$$

The weight of remaining grains in the cob after the shelling was calculated and then converted to kg h^{-1} by plant density.

Percent broken grains:

$$\%\text{ moisture} = \frac{\text{Grain weight before drying} - \text{grain weight after drying}}{\text{Grain weight before drying}} \times 100$$

Weight of broken grains: This was calculated by weighing the broken and incomplete grains and then converted to kg h^{-1}

Percent total loss: It rpresents the percentage of the remaining grains in the cob plus the broken grains.

Weight of total loss (kg h^{-1}): It represents the weight of the remaining grains in the cob and the broken grains.

Net grain yield (kg h^{-1}): It represents the total grain yield of clean and undamaged grains and then converted to kg h^{-1}

RESULTS AND DISCUSSION

Effect of varieties: There were no significant differences among the varieties and behaved similarly in terms of being affected by the other studied factors (ascorbic acid and harvest date) (Table 1). The average of moisture content in the grains was 23.63, 23.31 and 22.82% in Baghdad, Fajir, and Sara varieties, respectively. These varieties did not differ in the percentage of total grain loss and gave net grain yield of 6738, 6646 and 6695 kg h^{-1}, respectively. This may be due to the fact that the three varieties (Baghdad, Sara, and Fajir) were derived from the same origins and under the conditions of Iraq through selection.

Effect of ascorbic acid: The ascorbic acid had a significant effect on the broken grain percentage, broken grain weight, and net grain yield (Table 2). The spraying with ascorbic acid at 200 mg L^{-1} resulted in the highest broken grain percentage and broken grain weight (2.587% and 180.09 kg h^{-1}, respectively) while this treatment produced the highest net grain yield 6790 kg h^{-1}. This may be due to the role of ascorbic acid in the stimulating of biological processes, especially the processes of absorption by the plant (Hussein et al. 2011), building the shoot and root systems and dry weight (Atta Ullah et al 2016), stimulating many enzymes (Tedone 2004), conservation of chlorophyll, increase the rates of photosynthesis, and transfer dry matter to the grain (Shahnawas et al 2017). Darvishan et al (2013) indicated that spraying with ascorbic acid caused a significant increase in the yield. The increase of grain yield due to the ascorbic acid spraying may be attributed to the high the broken grain percentage and broken grain weight compared to control treatment. The percentage of moisture in the grain, the loss due to the weight of the remaining grains in the cobs after shelling and its percentage, the percentage of total loss, and the weight of the total lost grains were not significantly affected by the concentration of ascorbic acid.

Effect of harvest date: The early and late date of the harvest caused significant differences in the studied (Table 3). Moisture content was decreased from 31.40 to 16.27% when the harvest date was delayed from 30 days to 50 days with a decrease of more than 15%. The delay of harvesting led to increase grain loss by shelling, so percentage of the remaining grains in cob and weight was increased by harvesting early with 0.5248, 2.0922, and 3.4085%; and 39.98, 149.33, and 222.27 kg h^{-1} when harvested at date of 50, 40 and 30 days after pollination, respectively. The percentage of the broken grains weight, the percentage of total loss, and the total grain weight loss during shelling increased when the harvest date was delayed. But there was increase in the percentage of broken grain loss from 0.7719 to 3.563%, weight from 54.23 to 232.94 kg h^{-1}, the percentage of the total grain loss and its weight from 1.301 to 6.972% and from 94.21 to 457.02 kg h^{-1}, respectively when the harvest date was delayed from 30 to 50 days after pollination. The net grain yield decreased from 7203 to 6080 kg h^{-1} when harvesting early from 50 to 30 days after pollination, respectively. Patel and Varshney (2014) also showed that an increase in moisture content leads to increase the yield loss to a percentage that may reach more than 11%, and the appropriate time to harvest corn is at moisture content 20% or less (Mets 2006). Siddique and Wright (2003) and Samara et al. (2005) indicated that early harvest time caused higher moisture content, lower grain weight, lower grain yield, and higher broken grains during harvest and shelling. Vera et al. (2006) also observed that early harvest resulted in lower yields due to incomplete accumulation dry matter in grains.

Interaction between varieties × concentrations: There were significant interaction between varieties and ascorbic acid concentration in percentages of the broken grains, their weight, and the net grain yield (Table 4). In Baghdad × 200 mg L^{-1} broken grains percentage and weight was significantly higher (2.854% and 200.92 kg h^{-1}, respectively) and lowest was in Sara × 100 mg L^{-1} with (2.044%, 139.63 kg h^{-1}, respectively). This t indicates that interaction between the varieties and acid concentration in broken grains percentage, their weight, and net grain yield, had a different behavior. The difference of the varieties' response to the acid concentrations showed significant effect. There was no significant difference in the response of the varieties to ascorbic acid concentration in these traits.

Interaction between varieties × harvest date: The interaction between varieties and harvest date had a significant effect on the percent of total grain loss, and

Table 1. Effect of varieties Baghdad, Fajir and Sara on various traits

Varieties	% moisture	% remaining grain.ear^{-1}	Grain weight. ear^{-1} (gm)	% broken grain	Broken grain weight (kg h^{-1})	%total loss	Total weight loss kg h^{-1}	Net grain yield kg h^{-1}
Baghdad	23.63a	1.9467a	136.05a	2.477a	170.12a	4.4056a	308.041a	6738.55a
Sara	23.31a	1.9959a	134.70a	2.352a	159.33a	4.3533a	294.033	6646.06a
Fajir	22.82a	2.0830a	140.84a	2.351a	161.51a	4.4341a	302.352a	6695.28a

Table 2. Effect of ascorbic acid concentrations on various traits

Con. mg l^{-1}	% moisture	% remaining grain ear^{-1}	Grain weight. (gm)	% broken grain	Broken grain weight (kg h^{-1})	% total loss	Total weight loss kg h^{-1}	Net grain yield kg h^{-1}
0	23.33a	2.200a	149.66a	2.377ab	157.70b	4.564a	309.17a	6557.17a
100	23.05a	2.064a	140.36a	2.217b	153.18b	4.280a	293.17a	6731.75a
200	22.39a	1.761a	121.56a	2.587a	180.00a	4.348a	293.54a	6790.96a

Table 3. Effect of harvest date on various traits

Days after pollination	% moisture	% remaining grain ear^{-1}	Grain weight. (gm)	% broken grain	Broken grain weight (kg h^{-1})	% total loss	Total weight loss kg h^{-1}	Net grain yield kg h^{-1}
30	31.40a	3.4085a	222.27a	3.563a	222.27a	6.972a	457.02a	6080.55c
40	22.10b	2.0922b	149.33b	2.846b	149.33b	4.920b	353.19b	6795.40b
50	16.27c	0.5248c	39.98a	0.7719c	54.23c	1.301c	94.21c	7203.93a

Table 4. Effect of interaction between varieties × ascorbic acid concentration on various traits

Varieties	Concentration (mg l^{-1})	% moisture	% remaining grain.ear^{-1}	Grain weight. ear^{-1} (gm)	% broken grain	Broken grain weight (kg.h^{-1})	%total loss	Total weight loss kg.h^{-1}	Net grain yield kg.h^{-1}
Baghdad	0	24.58a	2.0222a	146.62a	2.252ab	147.81b	4.219a	299.88a	6683.5abc
	100	23.14a	2.3079a	157.79a	2.326ab	161.64ab	4.633a	319.43a	6738.0abc
	200	23.17a	1.5100a	103.73a	2.854a	200.92a	4.364a	304.81a	6794.2ab
Sarah	0	23.19a	2.2567a	151.01a	2.480ab	164.62ab	4.751a	315.63a	6472.2c
	100	23.91a	1.8411a	123.01a	2.044b	139.63b	4.885a	262.64a	6708.9abc
	200	22.83a	1.8900a	130.07a	2.533ab	173.75ab	4.432a	303.82a	6757.1abc
Fajir	0	22.21a	2.3220a	151.34a	2.400ab	160.67b	4.722a	312.01a	6515.0abc
	100	22.10a	2.0422a	140.28a	2.280ab	158.27ab	4.322a	298.56a	6748.3abc
	200	24.15a	1.8844a	130.89a	2.373ab	165.60ab	4.258a	296.49a	6821.6a

Table 6. Effect of interaction between ascorbic acid concentration × harvest date on various traits

Concentration (mg l^{-1})	Harvest date (days after pollination)	% moisture	% remaining grain.ear^{-1}	Grain weight. ear^{-1} (gm)	% broken grain	Broken grain weight (kg.h^{-1})	%total loss	Total weight loss kg.h^{-1}	Net grain yield kg.h^{-1}
0	30	31.56a	3.541a	2258a	3.777a	243.2a	7.318a	474.5a	5439.3d
	40	22.06b	2.411b	168.7bc	2.650b	186.1b	5.007b	354.8b	6650.9c
	50	16.35c	0.649c	54.4d	0.705c	43.7c	1.368c	98.17c	7081.3ab
100	30	31.57a	3.609a	235.5a	3.347a	221.0ab	6.956a	458.5a	6132.4d
	40	21.45b	2.042b	146.6c	2.622b	188.8b	4.664b	335.4b	6851.9bc
	50	16.12c	0.540c	36.9d	0.631c	49.7c	1.220c	86.61c	7210.9a
200	30	31.05a	3.075b	203.4ab	3.566a	234.5ab	6.642a	437.9a	6169.9d
	40	22.78b	1.823b	132.6c	3.265ab	236.5ab	5.089b	369.28b	6883.4bc
	50	16.33c	0.385c	28.6d	0.929c	69.2c	1.314c	97.86c	7319.6a

highest was 7.215% in Fajir × 30 days after the pollination There was with no significant difference interaction between two varieties (Baghdad and Sara) with the same harvest date (30 days after pollination).

Interaction between ascorbic acid concentration × harvest date: Interaction between the acid concentration and harvest date showed a significant difference (Table 6). The interaction 100 mg L^{-1} × 30 days after pollination was superior in of moisture percentage, the percentage of grains in ear and weight (31.57%, 3.609%, and 235.5 kg $^{-1}$, respectively). The interaction non-additive (0) × 30 days after pollination gave the higher percentage of broken grains, its weight, total loss percentage, and its weight, (3777%, 243.2 kg h^{-1}, 7188%, and 474.5 kg h^{-1}respectively) . There was significant effect of the harvest date on all traits and non-significant effect of the ascorbic acid concentration on moisture percentage, percentage of the remaining grains in the cobs and their weight, and the percentage of total loss and its weight. These traits were more affected by the harvest date. The significant effect of the ascorbic acid concentration on the traits of broken grain percentage, its weight, and net grain yield indicated that the factors of concentration and harvest date behaved differently.

CONCLUSION

The Baghdad variety was superior with under the effect of ascorbic concentration and different harvest date, and the opposite effect of the ascorbic acid on yield and on the reduction of yield loss on the early harvest date. The harvesting of corn plants cause increasing grain moisture, broken grain and yield loss.

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Effect of Organic Nutrient Sources and Chemical Fertilizers on Crop Growth and Yield of Sesame (*Sesamum indicum* L.) in Coastal Saline Zone of West Bengal

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***Abstract*:** Sesame has great potential in decreasing current gap between production and consumption of vegetable oil with lower levels of agricultural inputs. Combined application of various organic and inorganic nutrient sources is addressed by many researchers for obtaining higher yield of many oilseed crops. However, fewer findings are available on this to enhance growth and yield of oilseeds in Indian sub-continent. Field experiment was formulated to study the effect of different organic nutrient sources in combination with various doses of nitrogenous fertilizer for enhancing seed and oil yield of sesame in *pre-kharif* season of 2023 and 2024 comprising nine treatments combining different percentage of nitrogenous fertilizer, vermicompost, FYM, sulphur and *Azospirillum* that can significantly improve growth and yield of sesame. Application of 75% RDN + 25% vermicompost + *Azospirillum* recorded highest seed yield (1083 kg/ha) and oil yield (479.7 kg/ha) among all the treatments, although 75% RDN + 25% vermicompost + sulphur @20 kg/ha significantly increased oil content (44.7%) of sesame. Combined effect of vermicompost and microbial inoculation reflected better vegetative and reproductive growth that ultimately triggered seed and oil yield of sesame while improved oil content was obtained due to additional sulphur fertilization.

***Keywords*:** Sesame, Seed yield, Vermicompost, Microbial inoculation, Sulphur fertilization

Oilseed crops are thought to be the second most significant economic factor after cereals influencing India's agricultural economy. India has now become self-sufficient in cereal crop production while it is lagging behind in oilseed production. India is currently the world's biggest importer of vegetable oils despite being the fifth- largest nation in oilseed production. Therefore, it is crucial to maintain and increase the productivity of oilseed crops in order to sustain food and nutritional security (DRMR 2015, Meena et al., 2015, Lal 2016, Singh and Singh 2017 and Singh et al., 2017). In India, per capita vegetable oil consumption is 14.1 kg, which is substantially less than the global average of 23.6 kg (GOI 2015). Again, the current population growth is driving up per capita demand at a rate that domestic oilseed supply could not keep up with. Thus, there is an immediate need for augmentation of vegetable oil production in India.

Sesame (*Sesamum indicum* L.) is considered as an important edible oil source in terms of nutritional benefits as well as high quality oil in the world. It has great potential in decreasing the current gap between the production and consumption of the vegetable oil with lower levels of agricultural inputs. Sesame oil contains about 17-20% of good quality protein along with 40-50% of vegetable oil (Gholamhoseini 2020). However, majority of the cultivated area is occupied by small and marginal landholders with limited agronomic management. In contrast, the crop can be grown under limited water supply and fairly high temperature due to its relatively high drought tolerance with shortened vegetative growth.

Varieties with high yield potentiality, application of adequate amount of nutrients, weed control and good irrigation water management practices are the key to enhanced production of any crop. Similarly, productivity of sesame can also be enhanced following several agronomic measures including nutrient management practices. Application of various organic nutrient sources (manures, bio-fertilizers etc.) combined with chemical fertilizers is frequently addressed by many researchers for obtaining higher crop yield and improving soil biological, physical and chemical properties. The current tendency is to investigate the possibilities of using organic manures, which are more economical and environmentally benign, in addition to chemical fertilizers (Das and Goswami 2017). Application of bio-fertilizer can also significantly improve the growth and yield parameters of sesame. However, fewer findings are available on using various organic nutrient sources in combination with chemical fertilizers under field conditions to enhance the growth and yield of sesame in coastal soils of the Indian sub-continent. Therefore, this experiment was formulated to replace a part of nitrogenous fertilizers by organic nutrient sources for augmenting the crop yield, enhancement of economic benefits and improving the health of these poorly fertile coastal soils.

MATERIAL AND METHODS

Experimental site: The field experiment was carried out at The Neotia University, Diamond Harbour, South 24 Parganas, West Bengal in the *pre-kharif* season of 2023 and 2024. The farm is geographically located at 22°26' N latitude and 88°19' E longitude with an average altitude of 8 m above the mean sea level . The soil of the experimental field was fine textured and clayey type, having 213.6 kg/ha available N, 22.5 kg/ha available P_2O_5, 312.26 kg/ha available K_2O and 0.46% organic carbon. The climate of the area is sub-tropical and humid. The experimental period was hot and received moderate rainfall (Table 1).

Layout and treatment details: The experiment was carried out in a randomized complete block design (RCBD) comprising nine treatments each replicated thrice with plot size of 4 m × 3 m. Width of irrigation channels was 1 m and bund width of each plot was kept 0.5 m. Treatment details with different nutrient combinations are given in Table 2. FYM and vermicompost were collected from the Instructional Farm (dairy and vermicompost unit), School of Agriculture and Allied Sciences, The Neotia University and *Azospirillum* was brought from the Vivekananda Institute of Biotechnology, Nimpith Ashrama, South 24 Parganas, West Bengal.

Observation: Crop was raised as per recommended practices (Ranganatha et al., 2013). Agronomic observations were recorded from each plot by selecting ten plants randomly on plant height, branches per plant, dry matter accumulation and yield components *viz.* number of capsule per plant, capsule length, seeds per capsule, thousand seed weight, seed and stover yield and harvest index (HI) were taken. Approximately, 2 g of well dried sesame seeds were used from each treatment to estimate the oil content by Soxhlet apparatus using petroleum ether (AOAC 1960) and thereafter oil yield was calculated by multiplying the estimated oil percentage with the seed yield. Post-harvest soil samples were collected from each of the treatment plot from 0-30 cm soil depth. The samples were air-dried, crushed, sieved and stored in container for subsequent chemical analysis. The analysis involved all the standard procedures to determine available nitrogen, available phosphorus, and available potassium content as described by Jackson (1973). Soil nutrient index was also calculated (Parker et al., 1951) .

$$\text{Soil Nutrient Index (SNI)} = \frac{N_1 \times 1 + N_2 \times 2 + N_3 \times 3}{N_T}$$

Where,

N_1 = Number of samples falling in low class of nutrient status;

N_2 = Number of samples falling in medium class of nutrient status;

N_3 = Number of samples falling in high class of nutrient status and

N_T = Total number of samples.

Statistical analysis: Statistical data analysis was carried out using OPSTAT software and Fisher's least significant test was used to compare the mean values at a probability level of 0.05.

Table 2. Details of the experimental treatments

Treatment	Details
T_1	Control (RDF)
T_2	75% RDN + 25% Vermicompost
T_3	75% RDN + 25% Vermicompost + *Azospirillum*
T_4	75% RDN + 25% Vermicompost + Sulphur
T_5	50% RDN + 50% Vermicompost + *Azospirillum* + Sulphur
T_6	75% RDN + 25% FYM
T_7	75% RDN + 25% FYM + *Azospirillum*
T_8	75% RDN + 25% FYM + Sulphur
T_9	50% RDN + 50% FYM + *Azospirillum* + Sulphur

Table 1. Meteorological data during the period of field experimentation (pooled data)

Months	Temperature (^{0}C)		Rainfall (mm)	Relative humidity (%)		Bright sunshine (hrs)
	Maximum	Minimum		Maximum	Minimum	
February	29.72	18.42	6.8	92.46	63.37	6.83
March	33.61	21.35	32.2	93.85	65.42	7.12
April	36.45	24.81	15.4	94.75	66.13	6.75
May	39.42	25.87	18.7	94.21	61.43	6.63
June	36.12	24.78	51.2	95.16	58.19	6.23

Source: Department of Soil Science & Agricultural Chemistry, SAAS, TNU

RESULTS AND DISCUSSION

Crop growth parameters: Supplementation of inorganic fertilizer by organic nutrient sources in diverse combinations induced distinct variation in growth parameters of sesame to a great extent. Crop growth in terms of plant height increased as the crop grew older and moved towards maturity, irrespective of the difference in nutrient combinations (Table 3). Every aspect of nutrient variations positively impacted growth parameters. Amongst all the treatment combinations, the maximum height (99.2 cm) was in T_3 (*i.e.*75% RDN + 25% vermicompost + *Azospirillum*) followed by T_4 and T_2. Integrating chemical fertilizer, organic manure, and biofertilizer provided superior results in terms of nutrient availability and uptake as compared to the use of any of the three sources individually. Ahirwar et al., (2017) also reported that the trend of increase in plant height might be due to nutrient allocation from varied sources which aided in continuous availability of nutrients to the growing plants for a longer period. The significant reduction of plant height was when inorganic nitrogen was reduced to half of the recommended doses irrespective of biofertilizer and sulphur fertilization. The treatment of 75% RDN + 25% vermicompost along with additional application of either biofertilizer viz. *Azospirillum* or sulphur exhibited maximum primary branches production in both the seasons, whereas the treatment, half of the recommended doses of inorganic nitrogen together with any of the two different type of organic manure incorporation and supplementary sulphur and microbial inoculation i.e., T_5 and T_9 exhibited lowest number of primary branches per plant which might be due to lack of inorganic nitrogen that could enhance the rate of branch production. The incorporation of nutrients through organic sources especially via vermicompost and *Azospirillum* inoculation, along with 75% RDN and full P, K applications improved the dry biomass production per plant. At harvest, maximum amount of dry matter was produced from treatment T_3 (245.6 g/m^2) which was statistically at par with T_4, T_2 and T_1. Sahu et al., (2017) revealed that potential of free-living nitrogen-fixing bacteria to improve plant growth through nitrogen fixation as well as the impact of their metabolites secretion on the crop may be ascribed for the same when combined with potential biofertilizer or organic nutrient sources.

Yield attributes: The seed inoculation of sesame with *Azospirillium* in conjunction with 25% vermicompost + 75% RDN incorporation (T_3) significantly increased the capsule production in individual plant than remaining treatments (Table 3). Followed by the result of two consecutive years, the average variation of capsule numbers was to the tune of 20.48% from lowest (41.5) to highest (50.0) treatment. Integrated nutrient management through chemical fertilizer, microbial biofertilizer and vermicompost triggered better dry matter allocation to reproductive parts of plants. Veeral and Nayakanti (2019) also reported that balanced application of nutrients from different sources helped in greater photosynthetic activity and efficient translocation of photosynthates from source to sink, which might be attributed to greater number of capsule formations. Length of each capsule did not vary significantly due to variations of sources and doses of nutrients in respective seasons. The higher number of seeds per capsule was maximum (45.2) in T_3 followed by T_4, T_2 and T_1 which distinctly indicated that full recommended doses or partial (25%) replacement of chemical fertilizer + vermicompost in addition with *Azospirillium* or sulphur fertilization proved to be superior regarding seed production per capsule than FYM application along with the similar combinations. Diversified nutrient applications did not exhibit significant variations in 1000 seed weight of sesame plants.

Seed yield and oil yield: Seed yield revealed positive

Table 3. Effect of organic nutrient sources and chemical fertilizers on growth and yield attributes (pooled data)

Treatment	Plant height (cm)	No. of primary branches plant^{-1}	DMA (g m^{-2})	Number of capsule plant^{-1}	Capsule length (cm)	Number of seeds capsule^{-1}	1000 seed weight (g)
T_1	95.4	3.2	233.7	45.2	2.25	42.8	2.46
T_2	97.1	3.3	235.2	47.3	2.28	43.1	2.47
T_3	99.2	3.4	245.6	50.0	2.30	45.2	2.50
T_4	98.7	3.4	240.1	47.7	2.28	43.9	2.48
T_5	93.2	3.0	225.1	41.5	2.29	41.7	2.43
T_6	93.4	3.1	222.8	42.0	2.26	41.9	2.42
T_7	94.0	3.2	227.3	42.9	2.30	41.5	2.44
T_8	94.4	3.2	229.3	43.3	2.27	41.7	2.45
T_9	93.7	3.0	220.4	42.3	2.28	41.4	2.42
CD (p=0.05)	3.62	0.16	12.53	1.70	NS	1.63	NS

See Table 2 for treatment details

response due to nutrient supply from vermicompost, with supplemental application of *Azospirillum* inoculum or sulphur fertilization in combination with 75% recommended dose of nitrogen and 100% of phosphorous and potassium. Highest seed yield (1083 kg/ha) was from T_3 which was statistically at par with T_4 (Table 4). The combined effect of vermicompost and microbial inoculation reflected better vegetative and reproductive growth that ultimately helped in enhancing the seed yield. The increased seed yield of sesame under T_3 might be attributed to higher nutrient availability particularly N to the plants (Das et al., 2021). Simultaneous application of vermicompost and growth-promoting bacteria can also enhance the yield of sesame through a synergistic connection (Akhgar and Sotodeh 2021). Pooled over data of two consecutive seasons revealed that seasons T_3 recorded highest stover yield (3060 kg/ha) as compared to all other treatments. On the contrary, skip of half the recommended doses of inorganic nitrogen along with 25% FYM application with sulphur fertilization and microbial inoculation disclosed poor performance in respect to all the major parameters. No significant difference has been found for harvest index of sesame due to nutrient versatility. Highest oil content (44.7%) was recorded from T_4 treatment where sulphur fertilization was done additionally along with other inorganic and organic nutrient sources while T_3 treatment exhibited maximum oil yield (479.7 kg/ha) due to highest seed production. There was strong correlation of sesame seed yield with other growth and yield attributes during the study period (Fig. 1).

Available soil nutrients: Post harvest soil available N, P and K of sesame varied significantly within the treatments (Table 5). Treatments with full amount of N from urea solely or with vermicompost in combination with either microbial inoculation or sulphur fertilization recorded significantly higher soil available N over the treatments obtained nutrition from integrated application of inorganic fertilizers and FYM as organic nutrient sources. Significantly maximum available soil nitrogen (329.14 kg/ha) after harvest of the crop was in T_3 while lowest (271 kg/ha) was in T_9 treatment. Significantly higher available phosphorus (43.20 kg/ha) was in 100 % chemical fertilizer applied treatments. The maximum available potassium (353.16 kg/ha) content was registered from T_1 followed by T_3 and T_2. This could be because adding organic sources to the soil from vermicompost along with inorganic fertilizers generated an environment that was favourable for microbial multiplication and biochemical activity, enhanced water holding capacity and CEC of the soil led to a proliferating root system that added organic matter to the soil and released complex organic substances during organic matter decomposition, which in turn formed more stable available nutrients.

Nutrient index and soil fertility: Effect of multiple nutrient sources with versatile doses influenced the availability of soil nutrients significantly which in turn showed a distinct

Table 5. Effect of organic nutrient sources and chemical fertilizers on soil available nutrients (pooled data)

Treatment	Available nitrogen (kg/ha)	Available phosphorous (kg/ha)	Available potassium (kg/ha)
T_1	316.50	43.20	353.16
T_2	327.45	35.42	341.25
T_3	329.14	38.63	348.53
T_4	318.40	36.08	337.60
T_5	273.24	31.97	330.11
T_6	296.15	33.69	336.47
T_7	309.60	35.24	337.25
T_8	291.36	33.19	331.57
T_9	271.00	30.49	326.48
CD (p=0.05)	16.02	1.34	13.03

See Table 2 for treatment details

Table 4. Effect of organic nutrient sources and chemical fertilizers on yield and oil content (pooled data)

Treatment	Seed yield (kg/ha)	Stover yield (kg/ha)	Harvest index (%)	Oil content (%)	Oil yield (kg/ha)
T_1	952	2841	25.1	44.2	421.2
T_2	1029	3056	25.3	43.3	445.7
T_3	1083	3060	26.1	44.3	479.7
T_4	1056	3043	25.8	44.7	472.0
T_5	899	2748	24.7	43.5	392.2
T_6	963	2891	25.1	42.5	409.6
T_7	977	2863	25.5	41.6	407.2
T_8	981	2887	25.4	43.6	427.7
T_9	878	2646	24.9	41.3	362.6
CD (p=0.05)	46.09	163.51	NS	2.19	16.29

See Table 2 for treatment details

variation in nutrient indexing regarding all three macronutrients with special emphasis on nitrogen level (Table 6). SNI for available N, P and K was 1.78, 3 and 3 respectively, against the standard nutrient index value <1.67 for low, 1.67 to 2.33 for medium and >2.33 for high fertility status of the sesame harvested soil. Followed by the nutrient index analysis, soil fertility status is medium with respect to nitrogen content and status is high for both phosphorous and potassium content.

Production economics Application of 30 kg N (75% RDN) + 30 kg P_2O_5 (100% RDP) + 20 kg K_2O (100% RDK) through chemical fertilizers along with vermicompost and *Azospirillum* (T_3) inoculation as organic nutrient sources recorded maximum gross return (Rs. 89022 /ha), net return (Rs. 55837 /ha) and B:C ratio (1.68) (Table 7). The lowest

Table 6. Effect of organic nutrient sources and chemical fertilizers on soil nutrient index and fertility status (pooled data)

Available nutrients (kg/ha)	Nutrient index	Fertility status
Nitrogen	1.78	Medium
Phosphorous	3	High
Potassium	3	High

Table 7. Effect of organic nutrient sources and chemical fertilizers on production economics (pooled data)

Treatment	Cost of cultivation (Rs./ha)	Gross return (Rs./ha)	Net return (Rs./ha)	B:C Ratio
T_1	29293	77398	48102	1.64
T_2	33125	84584	51462	1.55
T_3	33185	89022	55837	1.68
T_4	33790	86803	53013	1.57
T_5	33850	73088	39244	1.15
T_6	31117	79158	48046	1.54
T_7	31177	80309	49134	1.57
T_8	31782	80638	48859	1.53
T_9	31842	71381	39538	1.24

See Table 2 for treatment details

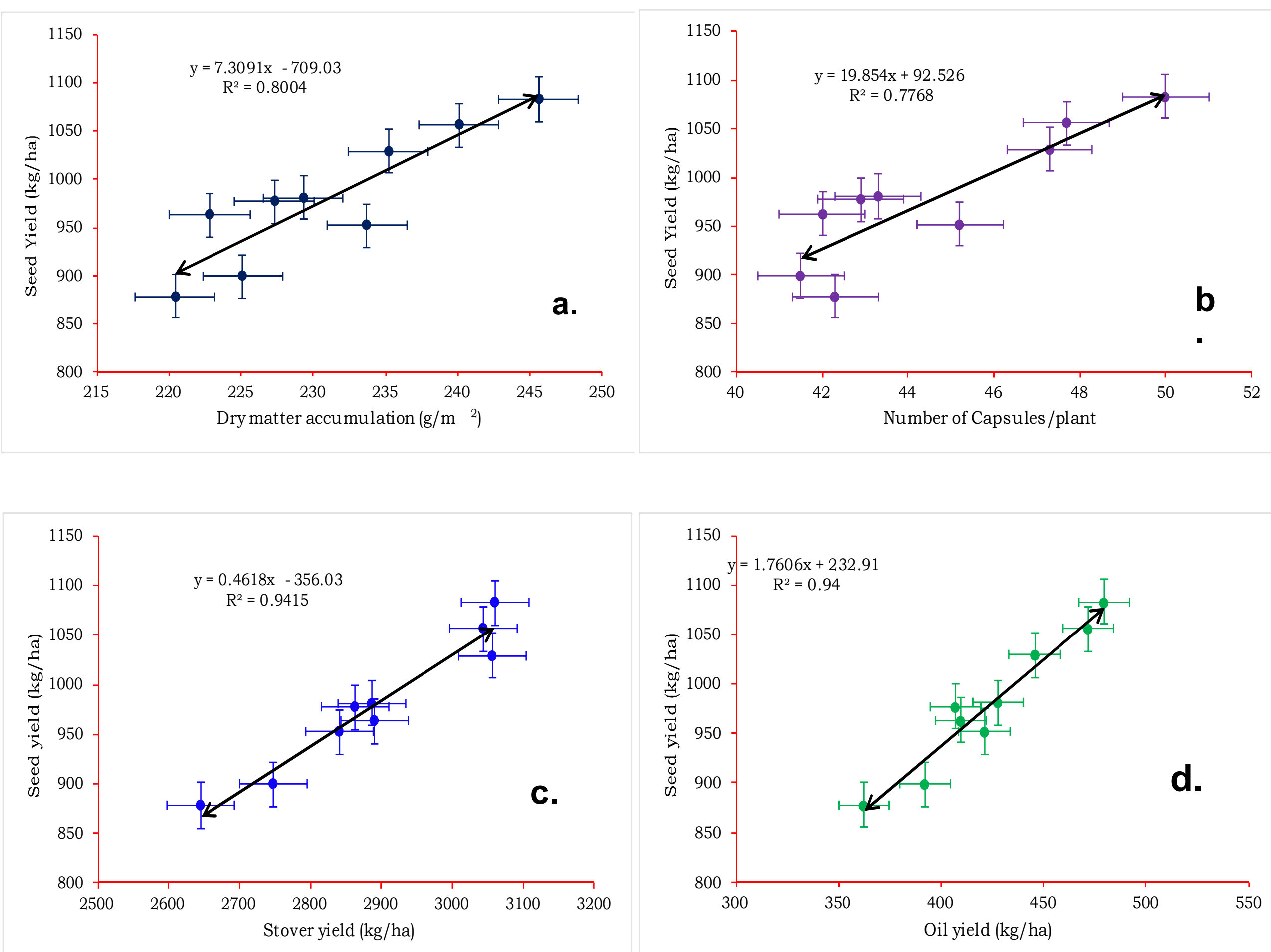

Fig. 1. Regression analysis of seed yield of sesame with a. dry matter accumulation, b. number of capsules $plant^{-1}$, c. stover yield and d. oil yield

monetary return in terms of net return and B:C ratio was obtained from T_5 where half amount of inorganic nitrogen was replaced by vermicompost incorporation followed by additional sulphur fertilization + microbial inoculation that might be attributed to high production cost.

CONCLUSIONS

The combined use of organic and inorganic sources of nutrients improved all the growth and yield attributes of sesame. Vermicompost and *Azospirillum* integration along with partial (25%) replacement of inorganic nitrogen fertilizer revealed highest seed and oil yield of sesame. Application of 75% RDN + 25% vermicompost + *Azospirillum* was identified as the best conjunction which exhibited higher content of available nitrogen in the soil and maximum economic benefit. Partial substitution of inorganic fertilizers with different organic nutrient sources not only offer better crop productivity but also strengthen soil health, thus maintaining the sustainability of eco-system to a great extent.

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Impact of Novel Bioformulations and Inorganic Fertilizers on Productivity of Soybean (*Glycine max* L. Merrill)

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Abstract: Experiment was carried out at Central Agricultural UniversityResearch farm, Andro, Imphal to evaluate the effect of the conjunction of inorganic fertilizers and novel bioformulations *viz.,* Bio NPK and Bio Zn with the recommended dose of fertilizers (RDF) on the growth and yield of soybean. The RDF was 20:60:40 kg/ha of N: P: K. The experiment was conducted in a randomized block design having seven treatments with a combination of RDF along with Bio NPK, Bio Zn and *Rhizobium japonicum* + MDSR 14 + 12c which was replicated thrice. The maximum yield (1564 kg/ha) was obtained in treatment 75% RDF + Bio-NPK followed by 75% RDF + Bio-Zn + Bio-NPK (1504 kg/ha) signifying that the yield improvement is possible with the conjoint application of biofertilizers and inorganic fertilizers. The maximum B: C ratio was in 75% RDF + Bio-NPK followed by 75% RDF + Bio-Zn + Bio-NPK.

Keywords: Bioformulation, Growth, Soybean, Yield

Soybean (*Glycine max* L. Merrill) is one of the most versatile oilseeds cum legumes grown all across the world, mostly for edible oil production. Manipur is one of the important soybean-growing states in the North-Eastern Hill region, the area, production and productivity of soybean are low compared to its potential. The estimated area, average yield and production of soybean for the agricultural year 2019-20 in Manipur were 0.042 lakh hectares, 870 kg/ha and 0.036 lakh tonnes , respectively (Directorate of Oilseeds Development 2019-20). Moreover, the favourable climate in Manipur makes it an ideal location for soybean production, especially in regions where paddy is unfit for cultivation (Sorokhaibam et al., 2022).

Soybean yields are generally low because it is a less prioritized crop, predominantly cultivated under rainfed conditions. Continuous depletion of soil nutrient resources due to intensive production has led to the emergence of multiple nutrient deficiencies in the soil, as higher and faster rates of nutrient exhaustion occur (Jain et al., 2021). There exists immense potential for enhancing soybean production through the application of organic manures, inorganic fertilizers, and biofertilizers (Verma et al., 2017). Although chemical fertilizers play a vital role in meeting the crop's nutrient requirements, persistent nutrient depletion poses a significant threat to sustainable agriculture. Consequently, there is an urgent need to reduce the usage of chemical fertilizers and instead increase the utilization of organic materials and other products. However, the sole use of organic inputs may not result in a spectacular increase in crop yield (Jain et al., 2021). Therefore, it is imperative to reduce chemical fertilizer usage and simultaneously increase the incorporation of organic and other sustainable products.

The importance of bioformulation in soybean cultivation and its potential impact on crop productivity is being explored worldwide. Bioformulations such as Bio-NPK liquid microbial consortium contain diverse populations of beneficial microbes, including nitrogen-fixing bacteria (*Azotobacter crococcum*), phosphorus-solubilizing bacteria (*Paenibacillus tylopilii*), and potassium-solubilizing bacteria (*Bacillus decolorationis*). Additionally, Bio-Zinc Liquid contains a single population of zinc-solubilizing bacteria (*Bacillus endophyticus*). These microbial inoculants aid in fulfilling the nutrient requirements of crops through efficient nitrogen fixation by promoting nodulation, solubilization of insoluble phosphorus, mobilization of potassium, and availability of zinc. Bioformulations play a vital role in enriching the soil by providing a range of essential nutrients, both micro and macro through nitrogen fixation, phosphate solubilization, and potash mobilization. Additionally, bioformulations aid in releasing substances that regulate plant growth, ensuring healthier and more productive crops (Javaid 2009). By utilizing these bio-inoculants, the nutrient demands of crops can be met through natural processes. The yield of soybean can improve yield to 5-10 % with the application of Bio-NPK and Bio-Zn bioformulations (Anonymous, 2020). Several studies have shown that bioformulation in soybean emerges as a promising strategy to enhance agricultural sustainability, improve crop yield, and minimize environmental impact.

MATERIAL AND METHODS

Field experiment was conducted during the *kharif* season in 2023 at Andro Research Farm (24°76' N and 94°05', 789 meters above mean sea level), Central Agricultural University, Imphal, Manipur to study the impact of integrating novel bioformulations and inorganic fertilizers on the growth and yield of soybean. The average maximum and minimum temperatures recorded during the growth period were 29.63°C and 20.30°C, respectively with an average rainfall of 72.04 mm and average sunshine of 5.82 hrs. The soil at the experimentation site was clayey in texture with a soil pH of 5.29 and 1.12% organic carbon content. However, the soil is low in available nitrogen, N (225.78 kg/ha) with a medium range of available phosphorus, P_2O_5 (23.29 kg/ha) and potassium, K_2O (265.43 kg/ha). The experiment was laid out in a randomized block design, with seven treatment combinations that were replicated thrice (Table 1). The NPK dose was applied in the form of urea, SSP and MOP @ 20:60:40 kg/ha as basal dose, respectively. The treatments i.e., bioformulations were applied as seed treatment as recommended. The tested variety was JS 97 52 and the crop was sown on 25th July and harvested on 11th November 2023. The observations on plant height at harvest (in cm), dry matter accumulation at 30, 45 and 60 DAS, number of nodules per plant at full bloom stage (R2 stage) and initial seed filling stage (R5 stage), number of branches per plant, number of pods per plant and yield were recorded. The economics of the crop is calculated as per the prevailing market price of soybean.

RESULTS AND DISCUSSIONS

Growth parameters: The significant difference in plant height at maturity was observed (Table 1). Maximum plant height (71.47 cm) was in T_5;75% RDF + Bio-NPK which was statistically at par with T_4; 75% RDF + Bio-Zn (67.27 cm) and T_6; 75% RDF + Bio-Zn + Bio-NPK (64.93 cm). The dry matter accumulation at 30 DAS showed no significant difference. However, at 45 and 60 DAS significance was observed (Table 1). At 45 and 60 DAS, maximum dry matter (2.12 g/plant, 15.57 g/plant) accumulated in T_5 which was statistically at par with T_6 (7.02 g/plant, 14.45 g/plant), T_2 (6.71g/plant, 13.97 g/plant) and T_7(6.36 g/plant), respectively. This might be due to the continuous supply of adequate nutrients during the crop growth period which has improved the plant height (Singh *et al.,* 2013) and the plants used these nutrients for vegetative growth which would have led to more dry matter accumulation as well. Similar findings were reported by Prajapat et al. (2015),Bondey et al. (2017), Kumar and Sharma (2018).

The number of nodules was observed at two important stages of soybean *viz.,* R2 stage and R5 stage which have shown significant influence of the treatments (Table 1). At the R2 stage, the maximum number of nodules was in T_6 which was statistically at par with T_4 and T_5. At the R5 stage, a significant maximum number of nodules was in T_5 (27.50) which was statistically at par with T_6(23.70), whereas the lowest number of nodules was in control at both stages. Applying bioformulations may have increased the microbial activity in the soil and enhanced the biochemical processes. The phosphorus solubilization helps in root development and builds a larger root network by providing more surface area to the nitrogen-fixing bacteria for the formation of the nodules and nutrient absorption (Salve and Gunjal 2011). Due to the presence of high organic matter in the soil, microbes gain a sufficient amount of energy for the mineralization of the nutrients (Singh et al., 2021). Thus, the biofertilizer treatments have a profound effect on nodule number (Jaga and Sharma, 2015). The findings are in agreement with

Table 1. Effect of treatments on growth parameters of soybean

Treatments	Plant height (cm)	Dry matter accumulation (g/plant)			No. of nodules/plant		No. of branches/ plant
		30 DAS	45 DAS	60 DAS	R2 stage	R5 stage	
T_1-Control	56.67	1.26	5.52	9.55	11.00	11.70	3.6
T_2-RDF	62.53	1.81	6.71	13.97	15.00	18.50	4.2
T_3-75% RDF	62.80	1.44	6.04	11.86	16.50	16.30	3.9
T_4-75% RDF + Bio Zn	67.27	1.85	6.07	12.06	20.20	16.50	4.1
T_5-75% RDF + Bio NPK	71.47	2.12	7.32	15.57	19.80	27.50	4.4
T_6-75% RDF + Bio Zn + Bio NPK	64.93	1.98	7.02	14.45	20.70	23.70	4.2
T_7-75% RDF + *Rhizobium japonicum* +MDSR 14 + 12c	60.87	1.80	6.36	12.16	16.70	20.80	4.0
Sem (±)	2.26	0.18	0.36	0.78	1.175	1.998	0.1
CD (p=0.05)	6.95	NS	1.11	2.41	3.621	6.155	0.4

RDF = 20:60:40 NPK kg/ha
75% RDF = 15:45:30 NPK kg/ha

Table 2. Yield attributes, yield and economics of soybean as influenced by different treatment combinations

Treatments	No. of pods/plant	Seed index (g)	Seed yield (kg/ha)	Straw yield (kg/ha)	Harvest index (%)	Net returns (Rs/ha)	B: C Ratio
T_1-Control	54	11.2	1045	2197	32.6	57219	1.55
T_2-RDF	76	11.4	1396	2691	34.1	76151	1.54
T_3-75% RDF	71	11.7	1229	2529	32.7	63024	1.33
T_4-75% RDF + Bio Zn	69	12.0	1197	2656	31	60066	1.26
T_5-75% RDF + Bio NPK	82	11.6	1564	2859	35.4	93066	1.95
T_6-75% RDF + Bio Zn + Bio NPK	79	11.7	1504	2766	35.2	87524	1.83
T_7-75% RDF + *Rhizobium japonicum* +MDSR 14 + 12c	74	12	1231	2605	33.2	63191	1.33
Sem (±)	3.19	0.48	70.66	106.8	1.84	6359	0.133
CD (p=0.05)	9.83	NS	217.73	329.1	NS	19595	0.409

RDF = 20:60:40 NPK kg/ha
75% RDF = 15:45:30 NPK kg/ha

earlier studies of Singh et al. (2021), Lakshman et al. (2023) and Somanagouda et al. (2023).The number of branches per plant was significant with a minimum number of branches in control treatment (3.6). In contrast, the maximum number of branches was in T_5 (4.4) which was statistically at par with the T_2 (4.2), T_6 (4.2) and T_7 (4.0) (Table 1). In the present study, a significantly higher number of branches are produced with the application of 75% NPK and Bio NPK due to the supply of balanced primary nutrients encouraging growth and development by cell division and elongation during the growth phase. These results are also in close conformity with Kumar and Sharma (2018).

Yield attributes and yield: The number of pods per plant was significantly influenced by the conjoint application of inorganic fertilizer and the bioformulations used. The maximum number of pods per plant was significantly higher in T_5;75% RDF + Bio-NPK (82) which was statistically at par with T_6; 75% RDF + Bio-Zn + Bio-NPK (79) and T_7; 75% RDF + *Rhizobium japonicum* + MDSR 14 + 12c (74) while the minimum number of pods per plant was in control (54) (Table 2). The combined application of inorganic fertilizers and Bio NPK has resulted in the release of nitrogen, phosphorus and potassium throughout the crop growth period and it has increased the dry matter accumulation and thereby increased the number of yield attributes along with good yield response. Similar results were observed in earlier studies (Bonde and Gawande 2017, Krevchenko *et al*., 2018, Shome *et al*. 2022).

Seed and straw yield were significantly influenced by the application of different bioformulation combinations (Table 2). The maximum yield was in T_5;75% RDF + Bio-NPK (1564 kg/ha seed yield and 2859 kg/ha straw yield) statistically at par with T_6; 75% RDF + Bio-Zn + Bio-NPK and T_2; 100% RDF. The lowest yield was recorded in the control. The inoculation of bioformulations with RDF has increased nitrogen fixation and the solubilization of P, K and Zn as well which has increased their availability for the plant uptake resulting in higher growth parameters and yield attributes which have then reflected in the higher seed and straw yield (Lakshman *et al*. 2023 and Singh *et al*. 2018, Gohil *et al*. 2021)).

However, the treatments have no significant effect on the harvest index and seed index of the crop (Table 2).The treatments might not influence the harvest index and seed index because of the varietal character and the less responsiveness to the treatments. The findings are in close agreement with Kumar and Sharma (2018) and Bonde and Gawande (2017).

Economics: The highest economic returns *viz.,* gross returns and B: C ratio were in T_5 (75% RDF + Bio-NPK) (Rs 93066/ha and 1.95) and statistically at par with T_6 (75% RDF + Bio-Zn + Bio-NPK) (Rs 87524/ha and 1.83) and T_2 (100% RDF) (Rs 76151/ha and 1.54).

CONCLUSION

Based on the aforementioned current observations may be concluded that the agronomic approach for optimum soybean growth, yield and economics could be better accomplished with the supplementation of 75% RDF + Bio NPK which was comparable with 75% RDF + Bio NPK + Bio Zn and 100% RDF.

AUTHOR'S CONTRIBUTION

Conceptualization and designing of the research work - TSD, AKB; Execution of field/lab experiments and data collection -TSD, AKB, SA, AK; Analysis of data and interpretation MJ, TO, RK; Preparation of manuscript AKB, TSD, SA.

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Genetic Diversity Based on Principal Component Analysis for yield and its Contributing Character in Linseed (*Linum usitatissimum* L.)

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Abstract: A field experiment was carried out to determine the principal component among eleven traits of linseed using 36 genotypes along with three checks. The data of eleven traits were recorded and analyzed to find out principal component to reveal the diversity among the linseed genotypes. the first five principal components exhibited more than one eigen values and consider for 70.60% of the total variation that consist of 20.56% (PC 1), 16.75% (PC 2), 11.87% (PC 3), 11.42% (PC 4) and 10.10% (PC 5) for eleven traits. PC I contributed maximum towards the variability (20.57%) followed by PC II (16.75%) and PC III (11.87%). The genotypes 180513 along PCA I axis and RL-15580 along PCA II axis identified on extreme positive side on both the axis were ponder to be the superior genotypes PC1 contributed the maximum towards the total variability (20.56%). The characters *viz.,* days to maturity, number of primary branches per plant, number of capsules per plant, number of seeds per capsule clarify the maximum variance in PC1. This study helps to the characterization, reduction of overlap in the data collection and evaluation of genetic diversity in linseed.

Keywords: Diversity, Eigen values, Linseed, Principal component analysis, Scatter plot

Linseed (*Linum usitatissimum* L.) is the most important *Rabi* oilseed crop after rape-seed and mustard, originates from Mediterranean and the south-west Asian regions Linseed is an annual herbaceous self-pollinated crop and belongs to the order Malpighiales, genus Linum and family *Linaceae*. The important linseed growing countries are India, Canada, China, USA, Russia, Egypt and Ethiopia. In India the area of linseed is (181 thousand ha) with production (41 thousand tones) and productivity (227 kg/ha), (Anonymous, 2020-2021). In terms of Rajasthan total area of growing linseed is nearby 5.7 thousand ha with production 6.1 thousand tones and productivity 1066 kg/ha. (Anonymous, 2019-20). The development of improved plant cultivars is restricted mainly due to narrow genetic pool, which results into limited possibility to restructure the linseed crop. Therefore, a technique is required for systematic reduction and summarization of data sets (Tanwar and Bisen 2017). The principal component analysis (PCA) was performed, which resulted to an effective contribution of different traits on the basis of respective variation. Evaluation of germplasm is helpful for both choosing a core collection and using it in breeding initiatives. PCA is superior over cluster analysis because it permits each genotype to be assigned into a single group (Mohammadi, 2002). The main objective of this research was to determine the potential genetic diversity and correlation by selecting parents for a hybridization programme based on PCA techniques in order to produce desirable segregants in the advanced generation.

MATERIAL AND METHODS

Thirty-six linseed genotype and three commercial checks (Pratap Alsi-1, Kota Barani-3, and Kota Barani-4) were obtained from the Agricultural Research Station, Kota and the Indian Institute of Pulse Research, Kanpur. These genotypes were grown randomized block design with three replications at College of Agriculture, Kota, during the *Rabi* season of 2019-20 situated at 234 meters above mean sea level on latitude 24º7' N and 76º62' E. Each genotype of linseed was sown in a 3 m long plot. The row to row and plant to plant distance was kept 20 cm and 10 cm, respectively. All required plant protection measures were taken to combat pests and diseases, and the suggested packages of practices were adhered to in order to raise a healthy crop (Sharma and Gupta, 2020). Observations were recorded on ten randomly selected plants per replication for eleven traits *(*Table 2*)* The scatter plot, correlation plot was performed using the package version 4.0.2 and principle component analysis by STAR (IRRI, 2014), XLSTAT (Addinsoft 2020).

RESULTS AND DISCUSSION

The first five PCs (PC1, PC2, PC3, PC4 and PC5) were responsible for 70.60 of the total variation. The first PC has the highest eigen value (2.27), explaining 20.56 percent of the variation (Table 1, Fig. 1). PCs with eigen values less than one were declared non-significant and so discarded since they are unlikely to have any practical significance (Mustafa et al., 2015). The majority of the variability in the set of all PCs

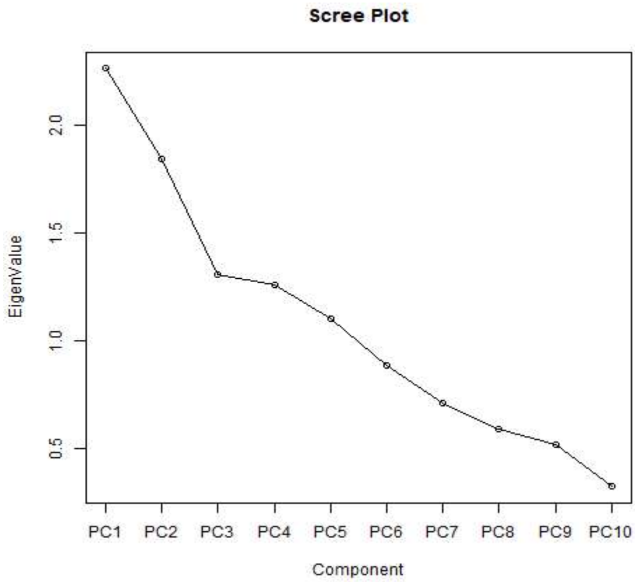

Fig. 1. Scree plot of 36 Linseed genotypes

was provided by PC1, while the remaining PCs tended to exhibit less variance.

Factor loading of various characters: The factor loadings of various variables computed *via* PCA. In the first PC, plant height, number of primary branches per plant, harvest index, 1000-seed weight and protein content had contributed greatest of the variation with positive significant coefficients of 0.61, 0.66, 0.51, 0.43 and 0.08 respectively. Seed yield per plant also showed positive coefficient which had contributed to the principal axis one and all remaining traits has negatively contributed to the principal axis one. High positive coefficients were for the number of primary branches per plant, capsules per plant and days 50% flowering in the second principal axis. Number of seed per capsule, harvest index and 1000-seed weight exhibited a negative impact on the second principal axis. In the third principal axis, days to maturity (0.45) , number of primary branches per plant (0.38) and plant height (0.32) had the highest co-efficient values,

Table 1. Eigen values and percent variation accounted for the 11 principle components of linseed genotypes

Statistics	Eigen values	Expression of proportion of variance	Expression of cumulative proportion
PC1	2.27	20.56%	20.56%
PC2	1.84	16.75%	37.31%
PC3	1.31	11.87%	49.18%
PC4	1.26	11.42%	60.60%
PC5	1.10	10.10%	70.60%
PC6	0.89	8.06%	78.67%
PC7	0.71	6.44%	85.10%
PC8	0.59	5.38%	90.48%
PC9	0.52	4.72%	95.20%
PC10	0.32	2.94%	98.14%
PC11	0.21	1.87%	100.00%

Table 2. Factor loading of eleven traits with respect to the significant principle component of linseed genotypes

Statistics	PC1	PC2	PC3	PC4	PC5
Days to 50% flowering	-0.37	0.42	-0.03	0.42	0.41
Days to maturity	-0.58	-0.06	0.45	0.43	-0.14
Plant height (cm)	0.61	-0.14	0.32	0.38	-0.05
Number of primary branches per plant	-0.01	0.73	0.38	-0.08	0.15
Number of capsules per plant	0.66	0.47	0.10	0.11	0.23
Number of seeds per capsule	-0.21	-0.72	0.10	-0.04	0.18
Harvest index (%)	0.51	-0.50	0.25	0.42	-0.07
1000 seed weight (g)	0.43	-0.18	0.20	-0.65	0.31
Protein content (%)	0.08	0.23	-0.44	0.01	-0.66
Oil content (%)	-0.15	-0.18	-0.63	0.23	0.52
Seed yield plant (g)	0.68	0.06	-0.38	0.28	0.04

whereas traits like oil content (-0.63), protein content (-0.44) and seed yield per plant (-0.38) shown highly negative contribution. In fourth principal axis, days to maturity (0.43), days to 50% flowering (0.42) and harvest index (0.42) had the highest co-efficient values, whereas traits 1000-seed weight shown highly negative contribution. In fifth principal axis, oil content, days to 50% flowering (0.41) and 1000-seed weight (0.31) had the highest co-efficient values, whereas traits

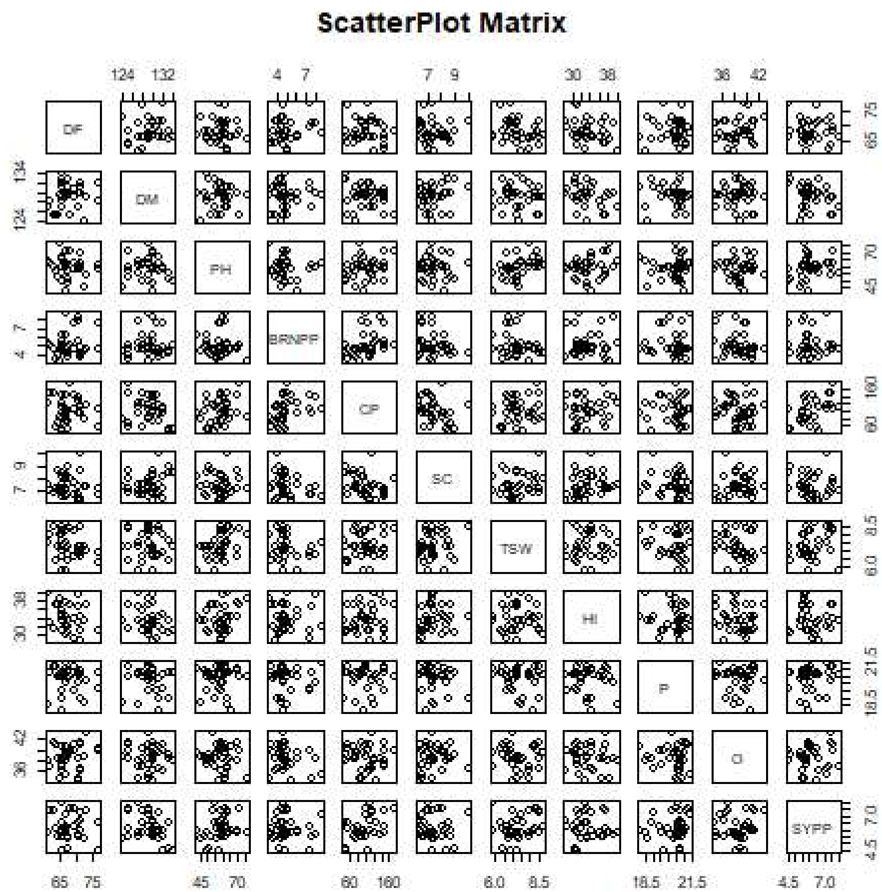

Fig. 2. Scatter plot with correlation value for eleven traits of 36 Linseed genotypes

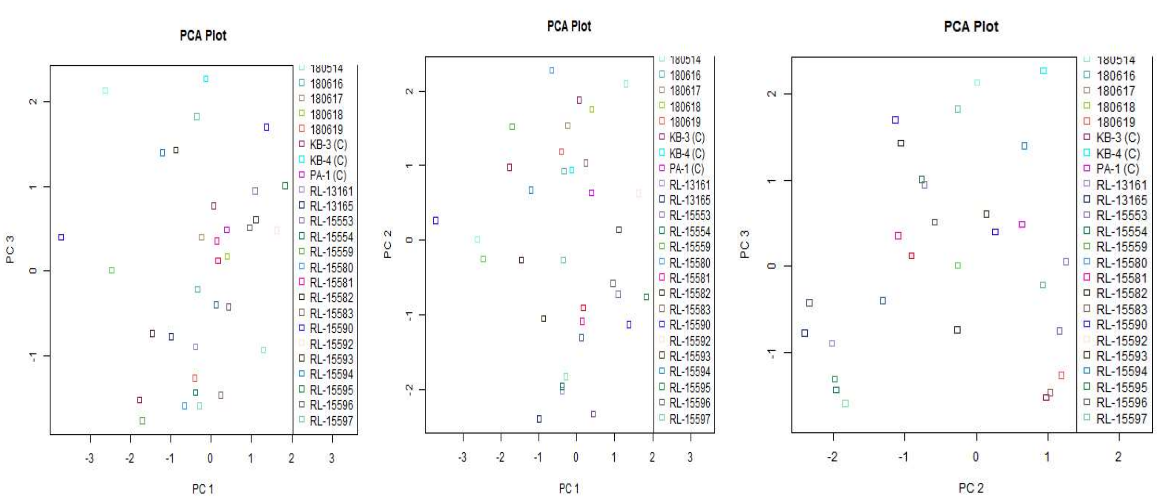

Fig. 3. Biplot of 36 genotypes of linseed on Principal Component axis I and II

protein content (-0.66) and days to maturity (-0.14) were shown highly negative contribution (Table 2, Fig. 2). The results on PCA indicated that these traits are important for trait manipulation and diversity in this population was present due to these traits. Similar trend was observed in earlier research (Singh et al., 2014, Kumar and Paul 2016, Rizvi et al., 2018 , Kumar and Kumar 2021.

Table 3. PCA scores of genotypes of linseed

Genotype	PC1 (X Vector)	PC2 (Y Vector)	PC3 (Z Vector)
180101	0.167	-0.905	0.120
180102	0.432	-2.337	-0.422
180103	0.117	-1.305	-0.397
180104	2.403	-1.978	-1.304
180107	-0.287	-1.830	-1.588
180203	-2.620	0.008	2.131
180204	-1.777	0.973	-1.521
180205	-2.464	-0.258	0.011
180512	3.349	1.249	0.054
180513	3.045	1.165	-0.750
180514	1.293	2.096	-0.928
180206	-3.715	0.264	0.398
180216	-1.456	-0.270	-0.738
180616	-0.337	0.928	-0.215
180617	-0.237	1.538	0.397
180618	0.395	1.751	0.172
180619	-0.409	1.184	-1.265
RL-15596	0.953	-0.583	0.514
RL-15597	-0.357	-0.264	1.821
RL-15595	1.831	-0.762	1.007
RL-15593	1.107	0.143	0.607
RL-15592	1.631	0.628	0.479
RL-15590	1.372	-1.133	1.697
RL-15553	1.085	-0.727	0.944
RL-15594	-1.205	0.672	1.395
RL-15581	0.143	-1.093	0.353
RL-15583	0.242	1.032	-1.466
RL-15580	-0.657	2.278	-1.590
RL-13161	-0.389	-2.019	-0.896
RL-13165	-0.994	-2.401	-0.777
RL-15554	-0.391	-1.960	-1.433
RL-15559	-1.707	1.521	-1.768
RL-15582	-0.877	-1.054	1.429
PA-1 (C)	0.382	0.637	0.489
KB-3 (C)	0.061	1.875	0.770
KB-4 (C)	-0.128	0.937	2.268

PC score of germplasm: The PCA scores for 36 linseed genotypes with three checks in the first three PCs were analyzed and considered three axes as X, Y and Z; and squared distance of each genotype from these three axes were computed (Table 3, Fig. 3). The genotypes identified on extreme positive side on both the axis were considered to be the best genotypes *i.e.* 180512 (3.349), 180513 (3.045), 180104 (2.403), RL-15595 (1.831), RL-15592 (1.631) and RL-15590 (1.372). These genotypes might be exploited in future breeding programs.

CONCLUSION

The study multivariate methodologies underscored the remarkable genetic diversity inherent in the utilized germplasm. Five out of the eleven principal components were significant (Eigen value >1) and accounted for 70.60 per cent of the variance, according to PCA. The yield and its contributing characteristics dominated in PC1 and PC2. Therefore, by utilizing heterosis, choosing germplasm with high PC1 and PC2 scores may lead to higher yield and yield characteristics. To develop superior high yielding lines, high performing material from each cluster can be used in a hybrid breeding program.

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Influence of Forcing Methods on Vegetative Growth of Kinnow Nursery under Arid-Submontane of Punjab India

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Abstract: The bud survival significantly affected by the time of forcing. Five and six weeks forcing periods were significantly superior to forcing immediately after budding. The scion growth bending method of forcing had better growth attributes in number of leaves, scion length and diameter than the rest forcing methods. In this study five weeks period of forcing increased percentage seedling survival, while this same treatment and four weeks forcing period reduced the nursery period of budling production. Bending method of forcing was best for improving bud survival and growth of Kinnow nursery plants.

Keywords: Bending, Forcing, Looping, Survival

The t-budding method of plant propagation is commercially used for kKinnow nursery plants multiplication in the spring and rainy seasons when the cambium is actively dividing (Carrol, 2017). It should be timed just before the spring season so that warm weather may help ensure a good bud union with root stock. Simultaneously, it should not be so late in autumn to avoid frost burn of the tender shoots of scion (Salik et al., 2015). In the semi-arid region of Punjab (Pakistan), the budding of sweet orange in the spring season gave the maximum budding success and survival (Salik et al., 2015). The demand for quality kinnow nursery plants is increasing, while the major hindrance is the 9-12 months' time period required for quality kinnow plants (Nawaj et al., 2021). The various practices viz; bending, cutting, tying, root training, and looping, are practiced for early production of nursery plants in the commercial nurseries (Neto et al., 2016). The bud forcing is a post-budding cultural operation used for the mass production of the saleable Kinnow nursery plants in a limited time (Thakur et al., 2017). Cutting off, looping or bending the citrus rootstock stem is a general practice practiced in fruit nurseries to force scion bud growth (Singh 2018). The best time of forcing is therefore necessary for a good Kinnow nursery development. Several studies were conducted in the past on Kinnow propagation at different times with the commercial method of propagation under varioust agro-ecological regions (Kumar et al., 2016), in sweet orange under the arid irrigated conditions of Haryana (Kanwar et al., 2015). Bhandari et al. (2022) in mid hills of Nepal. An intensive literature search did not reveal the best time of bud forcing operation in Kinnow nursery under the lower Shiwaliks of Punjab. Therefore, the present study was designed to find out the best time of post budding practice the bud forcing with minimum CLM damage for mass production of saleable Kinnow plants in a limited period.

MATERIAL AND METHODS

Location: The study was conducted in the enceptisol of the Regional Research Station, Ballowal Saunkhri, situated in the Lower Shivaliks with latitude of 31.092794° and longitude of 76.385244° (Fig. 1). The key features of this region include a sloping terrain, scattered small land holdings, and an irregular pattern of rainfall. The area experiences an annual rainfall of 982 mm, based on the average from 2015-19. The average monthly temperatures range between 2.9°C and 45.5°C. The sub-mountainous landscape of the Kandi region is characterized by loamy sand to sandy loam soils, which have limited moisture retention capacity. This soil composition makes dry farming agriculture in the region unpredictable and often chaotic. The lack of moisture retention in the soil poses challenges for sustaining crops, and the sloped landscape further complicates agricultural activities. Given the unpredictable nature of the rainfall and the specific soil characteristics, farmers in this region face challenges in optimizing agricultural practices. Understanding the site's conditions is crucial for contextualizing the study's findings and proposing practical solutions tailored to the unique challenges of the Lower Shivaliks.

Treatments detail: The field nursery experiments started on 12-month-old rough lemon rootstock seedlings that were budded with scions of Kinnow using the T-budding method.

Plant material, experiment design and treatments: The rootstock *JattiKhatti* (*Citrus jambhiri* L.) of the same age and size were selected and budded with the scions. The budding

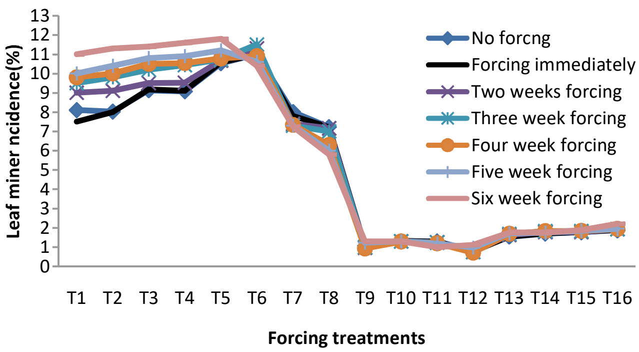

Fig. 1. Leaf miner infestation on different dates of budding and forcing method

instruments viz; budding knife, scissors, transparent plastic for wrapping and sun fiber for tying the scion and stock were used. The scion wood was prepared by clipping off the leaves, leaving petiole stubs 0.5 cm long intact. Bud wood, 10 cm in length, 9 to 12 months old**,** rounded with white streaks, was detached from the scion trees and grafted on 18-month-old seedling at a point having a T-shaped cut at a height 10-15 cm from the ground level. All the agronomic and cultural practices were kept constant and carried out regularly with all (Rattanpal et al., 2017). There 8 periods (time) of forcing and three forcing treatment methods were employed in factorial arrangement in randomized complete block design and replicated three times. The three forcing methods were: complete cutting back/topping (CCB), Looping/partial cutting back ½ - ⅔ of the stem and bending and tying (B/T). Data were collected on percent scion survival. Later growth recorded included number of leaves, scion length and diameter at two-week interval. T_1-T_{16} is weekly collection of percent infestation of CLM was recorded (Mustafa et al., 2014).

Statistical analysis: The mean of three duplicates was used to express the results. SAS 9.3 was used to analyze the experimental data.

RESULTS AND DISCUSSION

The forcing periods of five weeks, six weeks and no forcing influenced the percent bud survival over forcing immediately, seven weeks after budding (Table 1). Bending and tying method of forcing was superior in percent bud survival than the looping method of forcing. Five weeks of bending and tying produces highest (81.05 percent) bud survival than that of complete cut back and looping methods of forcing. There were interactions between forcing periods and method of forcing, especially for five five-week forcing periods, and the bending and tying method showed a significant effect on bud survival over other combinations (Table 1). Chahil et al. (2019) also observed a similar trend in citrus nursery. The scion length was significantly increased by delaying the time of forcing from fourteen weeks after budding; late forcing from four weeks after budding improves scion length (Table 2). The weeks forcing period was superior

Table 1. Effect of methods and time of forcing on percent (%) bud survival of kinnow nursery plants after seven weeks of budding

Time of forcing weeks	Method of forcing			Mean
	CCB	L	B/T	
No forcing	76.20	76.21	72.71	75.04
Forcing immediately	60.96	54.82	67.90	61.23
Two weeks	59.80	56.00	73.61	63.14
Three weeks	67.10	67.35	77.77	70.74
Four weeks	66.63	68.45	77.40	70.83
Five weeks	73.77	69.32	81.05	74.71
Six weeks	76.16	70.00	77.00	74.39
CV		17.20		17.95
CD (p=0.05)		6.89		11.52
Interaction forcing methods x time of forcing				
CV%		15.14		
CD (p=0.05)		10.38		

CCB = Complete cutting back, L = Looping, BT = Bending and tying

Table 2. Effect of time of forcing on scion length, diameter and number of leaves of Kinnow

Time of forcing weeks	Scion length			Scion diameter			Number of leaves		
	14	21	28	14	21	28	14	21	28
No forcing	21.87	27.21	33.56	0.29	0.30	0.41	10.21	16.45	21.45
Forcing immediately	18.23	23.59	30.14	0.27	0.27	0.29	11.45	17.82	20.18
Two weeks	22.47	27.92	35.83	0.29	0.37	0.43	10.45	22.65	28.31
Three weeks	24.21	29.42	40.23	0.29	0.41	0.54	10.76	26.94	36.72
Four weeks	29.22	37.72	44.12	0.28	0.45	0.55	12.84	25.15	35.25
Five weeks	33.58	39.25	46.14	0.28	0.46	0.63	13.94	26.31	38.73
Six weeks	30.94	36.72	42.15	0.29	0.45	0.56	11.84	24.54	34.83
CD (p=0.05)	5.85	8.04	3.18	NS	NS	0.21	NS	0.08	7.14

Table 3. Influence of different methods of forcing on the growth of Kinnow nursery plants

Time of forcing weeks	Scion length			Scion diameter			Number of leaves		
	14	21	28	14	21	28	14	21	28
Complete cutting back	20.46	26.49	36.18	0.23	0.36	0.50	14.14	18.32	22.71
Looping	22.16	28.83	37.43	0.25	0.42	0.55	14.05	17.58	20.42
Bending and tying	29.43	38.94	42.81	0.46	0.60	0.75	18.89	23.51	27.63
CD (p=0.05)	NS	7.12	3.08	0.18	0.12	0.11	2.58	4.78	2.83

Table 4. Effect of methods and time of forcing on scion length (cm) 28 weeks after budding in kinnow nursery plants

Time of forcing weeks	Method of forcing			Mean
	CCB	L	B/T	
No forcing	31.02	33.14	35.81	33.32
Forcing immediately	25.85	32.58	35.62	31.35
One week	32.87	34.56	38.92	35.45
Two weeks	30.45	34.86	40.85	35.39
Three weeks	37.73	35.14	47.49	40.12
Four weeks	45.10	38.52	48.43	44.02
Five weeks	48.15	43.82	47.56	46.51
Six weeks	38.19	44.63	46.48	43.10
CV		11.23		9.42
CD (p=0.05)		2.11		4.12
Interaction forcing methods x time of forcing				
CV%		1014		
CD (p=0.05)		4.96		

over the rest of the forcing periods. Scion diameter was influenced by time of forcing at twenty-eight weeks after budding; there were non-significant differences between no forcing, forcing immediately, one and two weeks forcing periods. Forcing time of five weeks enhanced scion diameter, twenty-eight weeks after budding than any of the above mentioned forcing periods. The number of leaves was also affected by delayed forcing periods, and the maximum number of leaves (38.73) was produced twenty eight weeks after budding (Table 2). Scion length, scion diameter, and number of leaves using the Bending and tying method of forcing were significantly superior to looping and topping /cutting back forcing methods (Table 3). Scion length was significantly higher after twenty-one weeks of different forcing methods and was maximum (38.94 cm) in the bending and tying method of forcing. Scion diameter and number of leaves recorded were significantly higher after fourteen weeks of various forcing methods. There were interactions among late forcing periods and the different methods of forcing after twenty-eight weeks of budding (Table 4). Five weeks and above had the highest bud survival because the healing process was completed while forcing before five weeks would disturb the healing process, resulting in death of the scion, therefore reducing scion percent survival. Bending and tying method supporting higher percent bud survival might have been caused by accumulation of specific carbohydrates and high indoleacetic acid (IAA) Adam (2016). Bending and tying methods favoured more availability of metabolites and higher accumulation of growth hormones that enhance bud survival. Late forcing time that enhanced the development of scion growth might be due to forcing periods supporting the production of carbohydrates by the citrus rootstock to support citrus scion growth (Chen et al., 2015). However, no forcing method or period led to competition between the rootstock and the newly produced scion, leading to a reduction of the growth of the scion. The bending and tying forcing method has the highest kinnow scion growth among the other two methods. Bending and tying forcing methods suppress growth of the citrus rootstock sprout while also supporting photosynthesis through its undisturbed plant parts.

The significantly higher incidence of leaf miner was recorded in three (6.12 %), four (5.22) and five weeks (6.0) of bud forcing after 7 days of budding, as compared to other treatments (Table 5). However, the incidence of leaf miner was non-significant in all the treatments after 28 days of budding. Overall, there was less than 7 per cent incidence of

Table 5. Effect of time of forcing on incidence of citrus leaf miner on Kinnow

Time of forcing weeks	Mean incidence of citrus leaf miner (%)		
	14	21	28
No forcing	3.72	2.00	1.34
Forcing immediately	4.34	2.52	1.58
One week	3.92	3.36	1.60
Two weeks	4.16	3.54	1.72
Three weeks	5.36	4.36	1.66
Four weeks	4.80	4.24	1.82
Five weeks	5.12	4.96	1.64
Six weeks	3.96	4.56	1.28
CD (p=0.05)	0.36	0.42	NS

citrus leaf miner. Ahmed et al.,. (2018) observed that increases in the incidence of *P. citrella* are usually due to the greater availability of leaf flushes and new shoots. Similar results were recorded by Ahmad et al., (2021) in citrus nursery production.

CONCLUSION

The forcing periods of four and above weeks improved the percent bud survival and scion growth of kinnow nursery plants. The superiority of a five-week forcing period on citrus scion development over early forcing periods made it the most preferred forcing period. The bending and tying forcing method also enhanced percent bud survival and scion growth more than cutting back and looping forcing methods. The vegetative growth and number of leaves affect the CLM population and can be used in future citrus IPM programs as a tool to suppress the CLM population.

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Phenological Responses of *Crataegus songarica* K Koch. to Climate Variation in the Kashmir Himalayas: Insights from Two Consecutive Years

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***Abstract*:** This study investigated the influence of temperature on the phenology of Hawthorn, *Crataegus songarica* K Koch. during 2021 and 2022. The phenological events consisting of bud set, bud bursting, leafing, flowering, fruit initiation, fruit fall, leaf tint and leaf fall, were observed during first year the bud set began in the third week of February, followed by bud bursting in the fourth week of March and in first week of April the leaves were fully open. Flowering started in the fourth week of April and continued until the fourth week of May, with the peak in second week of May. Fruit initiation was observed in the fourth week of July, while fruit fall started in the second week of September and lasted until the third week of October. Leaf tint was observed during the third week of September and leaf fall commenced in the fourth week of September, continuing until the first week of November. Investigations revealed that the entire phenophase cycle of *Crataegus songarica* lasted 9 months and 3 weeks. The observations indicated the early emergence of phenological events by 1 week in 2022 due to the higher temperature as compared to 2021. The entire phenophase cycle of *Crataegus songarica* in 2022 lasted 9 months and 3 weeks. By comparing these data of 2021 and 2022, can conclude the variations in the phenological patterns of *Crataegus songarica* and better understand the impact of environmental factors on its life cycle.

***Keywords*:** *Crataegus songarica*, Phenology, Temperature impact, Environmental factors, Climate change

The genus *Crataegus*, commonly known as hawthorn, comprises of a diverse array of species distributed throughout Asia, Europe and North America (Alirezalu et al., 2018). Among these species, *Crataegus songarica*, commonly, holds particular significance due to wide adoptability (Haq 2012, Rafeeq et al., 2023). It is a shrubby tree reaching heights of 4-5 meters with distinctive bark and foliage characteristics, occupies diverse habitats across Afghanistan, Iran, northern India, northern Pakistan and neighboring regions (Zaurov et al., 2012). In India, grow in temperate Himalayas of Kashmir and Himachal Pradesh, particularly in river valleys and ravine slopes at altitudes of 1800-3000 meters above sea level (Rafeeq et al., 2022). Phenology, the study of cyclic life events in plants and their correlation with seasonal and climatic variations, offers insights into the impacts of climate change (Chhetri et al., 2020). With global temperatures on the rise, understanding phenological shifts becomes imperative. Plant phenology, influenced by environmental factors, serves as a sensitive indicator of ecosystem responses to climate change (Kushwaha et al., 2011). The effects of temperature on the timing of phenological events, which hold implications for species distribution and ecosystem dynamics need special attension(Marques et al., 2004). Given the significance of phenological research in relation withaltering temperature, this study focuses on investigating the phenology of *Crataegus songarica* in the Kashmir Himalayas. The aim is to assess impact of temperature variations on key phenological events.

MATERIAL AND METHODS

Study area: Phenological studies of *Crataegus songarica* were conducted at Malhar, Ganderbal of Sindh Forest Division, to document the phenological events of the species and generating baseline data for further research. The study site, located at an altitude of 1850 m with geographic coordinates 34.33° N and 74.46° E, provided an ideal environment for observing the growth phases of *Crataegus songarica*.

Data collection: It was carried out using a systematic sampling approach. A total of 5 trees were randomly selected within the study site, and from each tree, 4 branches were chosen for observation. This sampling strategy ensured a representative sample of the population. Observations were conducted at weekly intervals, starting from February. This frequent sampling allowed for the accurate tracking of phenological events over time. Descriptive statistics and phenological indices were employed to interpret the data and identify any significant patterns or trends in the phenological cycle of *Crataegus songarica.*To capture the various stages of growth and development, a comprehensive set of parameters, including bud set, bud burst/break, leaf initiation/flush, flowering, fruit formation, fruit/seed fall, leaf tint, leaf fall and fruit fall was recorded

RESULTS AND DISCUSSION

The bud set occurred in the third week of February, followed by bud bursting in the fourth week of March, 2021. By the first week of April, the leaves were fully open, and flowering commenced in the fourth week of April, lasting until the fourth week of May, with the peak occurring in the second week of May. Fruiting initiation was observed in the fourth week of July, while fruit fall started in the second week of September and lasted until the third week of October. Leaf tint appeared in the third week of September, and leaf fall began in the fourth week of September, continuing until the first week of November. The entire phenophase cycle of *Crataegus songarica* in 2021 lasted 9 months and 3 weeks. The observations from 2022 indicate that bud set began in the second week of February, followed by bud bursting in the third week of March. By the fourth week of March, the leaves were fully open, and flowering commenced in the third week of April, lasting until the third week of May, with the peak occurring in the first week of May. Fruiting initiation was observed in the third week of July, while fruit fall started in the first week of September and lasted until the third week of October. Leaf tint appeared in the second week of September and leaf fall began in the third week of September, continuing until the fourth week of October. The entire phenophase cycle of *Crataegus songarica* in 2022 lasted 9 months and 3 weeks. By comparing these two years, understand the impact of temperature on variations in the phenological patterns of *Crataegus songarica* (Table 1, Fig. 2).

Temperature is a critical environmental variable that significantly influences tree phenology by regulating the timing of phenophases such as bud burst, leaf emergence, flowering, fruiting, leaf senescence, and leaf fall (Parmesan 2007). These phenological changes are closely linked to ecosystem functioning and the adaptation of species to their environments. Several studies have demonstrated that temperature variations can cause shifts in phenological events, leading to ecological and evolutionary implications (Parmesan 2007, Cleland et al., 2007). The findings corroborate these observations, as we documented an early emergence of phenological events in *Crataegus songarica* in 2022 compared to 2021. This shift is likely attributable to elevated temperatures during the phonologically active periods. Such early occurrences can have cascading effects on ecological interactions, such as pollination dynamics, seed dispersal and competition for resources among co-occurring species (Walther et al., 2002). The early emergence of phenophases in *Crataegus songarica* highlights the species sensitivity to temperature changes, which could have broader implications for its ecological fitness and distribution. Warmer temperatures may enhance or disrupt phenological synchrony with other organisms, such as pollinators or seed dispersers. Long-term monitoring and modeling are crucial to understanding how temperature-

Table 1. Phenophases of *Crataegus songarica* in Kashmir Himalayas

Phenological characteristic		Year 2021	Year 2022
Bud set		February (3rd week)	February (2nd week)
Bud burst/ break		March (4th week)	March (3rd week)
Leaf initiation/flush		April (1st week)	March (4th week)
Flowering	Initiation	April (4th week)	April (3rd week)
	Peak	May (2nd week)	May (1st week)
	Completion	May (4th week)	May (3rd week)
Fruit Formation/Seed set		July (4th week)	July (3rd week)
Fruit/seed fall	Initiation	September (2nd week)	September (1st week)
	Completion	October (3rd week)	October (3rd week)
Leaf tint		September (3rd week)	September (2nd week)
Leaf fall	Initiation	September (4th week)	September (3rd week)
	Completion	November (1st week)	October (4th week)

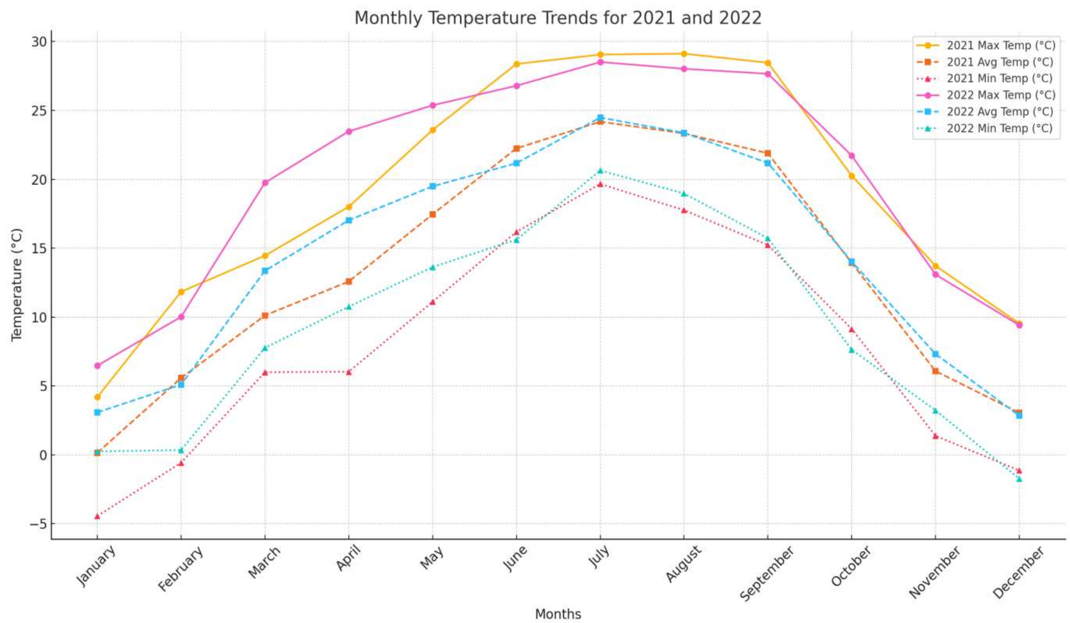

Fig. 1. Monthly average temperature of study area

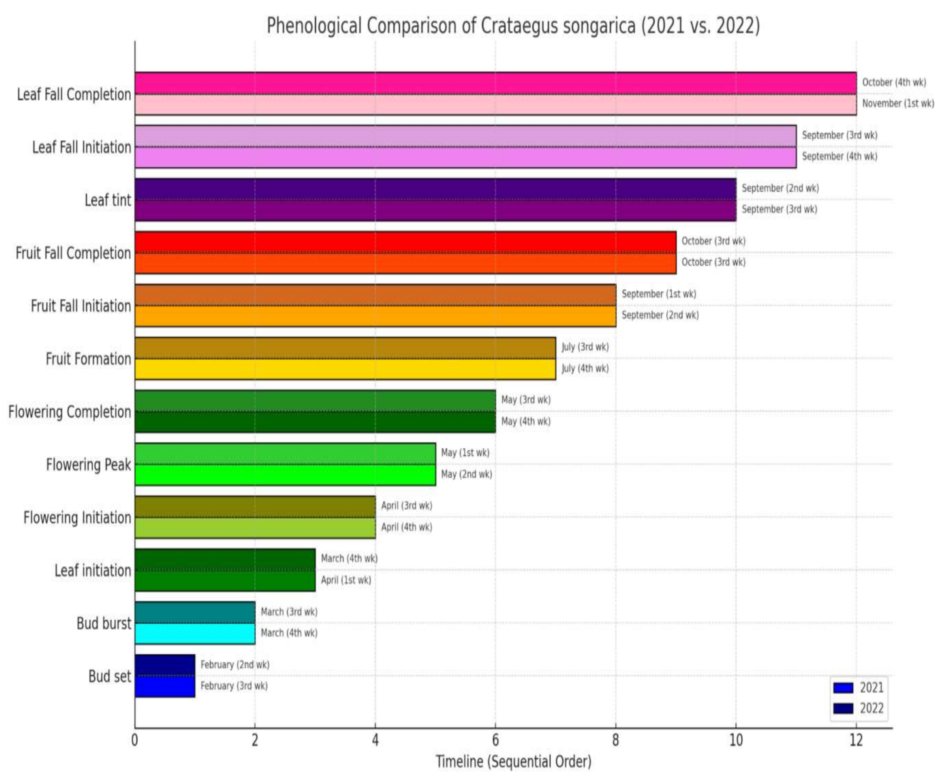

Fig. 2. Phenological comparison of *Crataegus songarica* 2021 vs. 2022

driven phenological shifts which may impact species survival, forest productivity, and ecosystem resilience (Cleland et al., 2007).Several researchers have also conducted studies on tree phenology with similar trend (Kumar et al., 2009, Mir et al., 2016, Singh and Negi 2018).

CONCLUSION

This study on phenology of Hawthorn across two consecutive yearsoffers valuable insights into the influence of temperature on its life cycle. The observed phenological events, from bud set to leaf fall, revealed a consistent pattern

Bud set **Bud burst** **Leaf initiation**

Flowering **Fruit formation** **Fruit/seed fall**

Leaf tint **Leaf fall**

Plate I. Phenophases of *Crataegus songarica*

across both years, with early occurrence of events in 2022 by one week, attributed to higher temperatures. This advancement underscores the sensitivity of *Crataegus songarica* to environmental changes, particularly temperature variations..

AUTHORS CONTRIBUTION

JR, KNQ, PAK, JAM and AS conducted conceptualized the study and conducted Phenological data collection and analysis. IAP, TM, MIJ, OAY and SF contributed in ecological interpretation and manuscript development and refinement.

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Diversity and Population Dynamics of Spiders in Spring and *Kharif* Maize (*Zea mays*) Crops of Punjab

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Abstract: The present study investigated the occurrence of 15 spider species, belonging to families Tetragnathidae, Araneidae, Oxyopidae, Lycosidae, Salticidae, and Araneidae in spring and 20 spider species of families Oxyopidae, Lycosidae, Salticidae, Pisauridae, and Phylodromidae in *kharif* maize crop. Spiders of family Lycosidae (40.0-53.33%) and genus *Pardosa* (nine species) were the predominant of all. In spring maize, higher spider population was during May (3.57 individuals/plot) with *Pardosa amenata* (56.51 individuals/plot) predominating significantly as compared to other spider species. In *kharif* maize, a higher spider population was recorded during August (2.76 individuals/plot) after which the spider population starts declining. *Lycosa pseudoannulata* (44.5 individuals/plot) and *Pardosa milvinia* (40.88 individuals/plot) predominated significantly as compared to other spider species. Increase in the Shannon-Wiener index, species richness and decrease in species evenness and Simpson's index was recorded in *kharif* as compared to spring maize crop. The higher spider population (29.34%) was in spring maize crop during May as compared to that in August in the *Kharif* maize crop. The study concludes that maize is a good host for the survival of spiders.Hence, pest management strategies, especially the use of pesticides should be used with caution during these times.

Keywords: Diversity, *kharif* maize, Population dynamics, Spider, Spring maize

Spiders are dominant arthropod predators in all ecosystems providing different ecosystem services, acting as models to evaluate ecological and evolutionary hypotheses (Michalco et al., 2019, Lowe et al., 2020) andact as biological indicators by responding differently to natural and anthropogenic activities (Rajeevan et al., 2019). Spiders act as biocontrol agents due to their potential for competition, catching more than consumption, intraguild predation, and resistance against starvation and desiccation (Sumitha and SudhiKumar 2020). They act as sources of molecules that may be useful for biotechnology and the development of medicines (Matavel et al., 2016). Spider venom contains more than ten million bioactive peptides which leadto the development of drugs against a wide range of pathophysiological conditions for the betterment of mankind (Prakash et al., 2023).

Globally, 52,590 spider species have been described which belong to 4,376 genera and 134 families (World Spider Catalogue 2024). Indian spider diversity constitutes about 1,971 species belonging to 500 genera under 61 families and constitutes about 3.79% of the total world's spider diversity (Sen et al., 2024). Spiders have been distributed almost in every ecosystem except Antarctica and can survive in the most extreme environmental conditions. Their evolution has led to a change in their morphological and behavioral characteristics, which allows multiple species to coexist within an ecosystem leading to vertical spatial distribution, due to which a variety of species can inhabit different niches throughout the same habitat (Yadav et al., 2005). Some species of spiders build webs to trap and kill the prey, and some actively seek out or ambush their prey (Selifa and Ganesh 2020). Species found in temperate climates adapt to significant temperature fluctuations throughout the year (Bukhari et al., 2012). Species belonging to Philodromidae and Clubionidae families have adapted to sub-zero winter temperatures by reducing the supercooling point of their hemolymph through the production of specialized proteins and glycerol, whereas in hot climates spiders can also regulate their internal temperature (Raghul and Kumar 2021).

Agricultural ecosystemspossess high spider diversity as compared to the natural ecosystem and it regulates terrestrial arthropod populations in different crops (Mathew et al., 2014). Spiders consume a variety of insect pests in different agricultural crops, reduce pesticide load in the environment, play a valuable role in pest management without causing damage to crops (Raji et al., 2024) and thus help boosting the economy of farmers. Many surveys have been conducted in different terrestrial areas and agroecosystems to estimate spider diversity (Dave and Trivedi 2024). However, there is a gap regarding the diversity of spiders in crops which vary from place to place. The present work intends to study the abundance and diversity of spiders from spring and *Kharif* maize crops in Punjab.

MATERIAL AND METHODS

Study area: The present study was carried out at maize crop fields of the Department of Plant Breeding & Genetics (Maize Section) and Department of Zoology, Punjab Agricultural University, Ludhiana on both *Kharif* and spring maize crops in2022. The selected experimental fields were prepared as per the Package and Practices for maize crops (Anonymous 2022) and were leveled and divided into plots according to the layout plan. The maize varieties selected were PMH-13 for *Kharif* and PMH-10 for spring crops, respectively, and sown in plots (each of size 8 x 3 m) with a planting distance of 20 x 60 cm (plant-plant and row-row). The spring maize crop was sown on February 17, 2022 and harvesting was completed on June 22, 2022. Similarly, the *Kharif* maize crop was sown on May 26, 2021 and its harvesting was done on September 27, 2021. Both *Kharif* and spring maize crops were raised using agronomic practices as recommended by the Package of Practices, Punjab Agricultural University, Ludhiana. No pesticides were sprayed in the selected plots.

Abundance and dynamics of spider population: Spiders were counted from the whole maize plant (leaves/stem) by visual searching method from randomly selected five blocks/plots covering all the geological sites as well as the center of the field at fortnight intervals. The size of each block was 1×1m and one plot (8×3 m) represented one replication, each plot having three replications. Spiders were counted on the plants and soil surface of both *kharif* and spring maize crops, respectively during morning hours (7.00-9.00am). Some samples of spiders were collected using hand-picking method for identification using suitable keys and confirmed by experts fromthe Zoological Survey of India, Calcutta. The collected spiders were transferred into glass vials having 70% alcohol.

Calculation of various indices

Relative abundance: The following formula was used to measure the percentage of individuals of a particular species:

RA = ni/N × 100

where, RA= Relative abundance

n_i = Total number of individuals in a particular sample, N = Total population of all species

Biodiversity indices: To calculate biodiversity indices, the following formulae were used:

Simpson index: $D = \Sigma n_i(n_i-1) / N(N-1)$

where, D = Simpson index, n_i = Total number of individuals in a particular species, N = Total population of all species

Shannon-Wiener index:

$H = \sum[(pi)\times\ln(pi)]$

pi = Proportion of total sample represented by species, where H' = Shannon-Wiener index, n_i = Total number of individuals in a particular sample, N = Total population of all species

Species evenness:

$$J' = \frac{H}{\ln(S)}$$

Where, J' = Species evenness, H= Shannon-Wiener index

Statistical analysis: Different indices like relative abundance, Shannon diversity index, species evenness, and species dominance were calculated.

RESULTS AND DISCUSSION

Diversity of spiders in *kharif* and spring maize crops: Results from fortnight surveys in spring maize crops revealed the occurrence of 15 different spider species belonging to five families namely Tetragnathidae, Araneidae, Oxyopidae, Lycosidae and Salticidae. The family Lycosidae accounted forhigher dominance (53.33%) by including eight spider species followed by Tetragnathidae, Oxyopidae and Salticidae (each having 13.33% dominance) whereas, family Areneidae accounted as of lowest dominance (6.66%) as compared to others (Table 1). In the family Lycosidae, spiders of genus *Pardosa* (nine species) predominate in both *kharif* and spring maize crops.

Similarly, in *Kharif* crop, 20 different spider species belonging to six families namely Araneidae, Oxyopidae, Lycosidae, Salticidae, Pisauridae and Phylodromidae were recorded. Among all those families, Lycosidae (40.0%) showed the highest dominance which included eight spider species followed by Areneidae and Oxyopidae (20.0%) each having four spider species whereas Pisauridae and Phylodromidae accounted as of lowest (5.0%) dominance (Table 1). The Shannon-Wiener index, species evenness, species richness, and Simpson's index for spider population were s 2.29, 0.84, 15, 0.12 and 2.411, 0.81, 20, 0.11 in spring and *Kharif* maize crops, respectively. There was a slight increase in the Shannon-Wiener index and species richness whereas a slight decrease in species evenness and Simpson's index was recorded.

Population dynamics of spiders in spring maize crop: In spring maize crop, all the spider species showed their first appearance during 1st fortnight of March whereas the appearance of spider species namely *Pardosa monticola, Oxyopes javanus, Oxyopes birmanica, Thyene imperalis, Leucage argyra* and *Telamonia dimidiate* was recorded during 2nd fortnight of March. The first appearance of *Trochosa terricola* was during 1st fortnight of April, whereas

Metepeiro labyrinthea and *Tetragnatha versicolor* were recorded during 2nd fortnight of April. Among all the recorded spider species only *M. labyrinthea* was not recorded one month before harvesting of spring maize crop. The increasing trend of spider population was recorded from March to June, however, a higher spider population was during 1st fortnight of May (3.7 individuals/plot) after which the spider population started declining. During June, due to the harvesting stage of the maize crop, the lowest spider population was recorded (2.02 individuals/plot). During the overall crop season of spring maize crop, *Pardosa amenata* (56.51 individuals/plot) predominated significantly followed by *Lycosa pseudoannulata* (42.57 individuals/plot) and *Pardosa pseudoannulata* (28.38 individuals/plot) as compared to other spider species.

Population dynamics of spiders in *kharif* maize crop: Mostly, all the spider species showed their first appearance during 2nd fortnight of June whereas the appearance of species *O. birmanica, Neoscona mukerjei,* and *Neoscona nautical* was during 1st fortnight of July. However, the first appearance of *T. imperalis* and *Gea theridiodes* was recorded during the 2nd fortnight of July. Also, among all spider species only *T. imperalis* was not recorded one month before the harvesting of *kharif* maize crop. The increasing trend of spider population was from June to July, however, a higher spider population was during 1st fortnight of August (2.92 individuals/plot) after which the spider population started declining. During September, due tothe harvesting stageof maize crop and change in environmental conditions, lowest spider population was recorded (1.48 individuals/plot). During the overall crop season of *kharif* maize crop, *L. pseudoannulata* (44.5 individuals/plot) and *Pardosa milvinia* (40.88 individuals/plot) predominated significantly followed by *P. pseudoannulata, Pirata piraticus, P. amenata* and *Pardosa agricola* (19.44-21.85 individuals/plot) as compared to other spider species.

Overall29.34% higher spider population was in the spring maize crop during May as compared to August of the *kharif* maize crop due to congenial environmental conditions. *P. amenata* was the predominant species in the spring maize crop but its population declined by 2.77 times in the *kharif* maize crop. Similarly, an increase in *P. milvinia* (2.04 times),*L. pseudoannulata* (1.04 times) and *P. pseudoannulata* population (1.05 times) was in the *kharif* maize crop as compared to the spring maize crop. Interestingly, among all spider species, eight species namely *Pardosa pravitaga, Pardosa uintana, Pardosa monticola, Leucauge argyra, Telamonia dimidiate, Metepeira labyrinthea, T. terricola, T. versicolor* were from spring maize but not from *kharif* maize crop. These species may have shifted to surrounding rice crop sown during June. Similarly, 14 spider species namely *Pirata piraticus, P.agricola,*

Table 1. Dominance of spider species recorded in spring maize crop

Family	Scientific name	% Dominance (Family)
Tetragnathidae	*Leucauge argyra*	13.33
Tetragnathidae	*Tetragnatha versicolor*	
Araneidae	*Metepeira labyrinthea*	6.66
Oxyopidae	*Oxyopes birmanicus*	13.33
Oxyopidae	*Oxyopes javanus*	
Lycosidae	*Pardosa amenata*	53.33
Lycosidae	*Pardosa milvinia*	
Lycosidae	*Pardosa monticola*	
Lycosidae	*Pardosa pravitaga*	
Lycosidae	*Pardosa pseudoannulata*	
Lycosidae	*Pardosa uintana*	
Lycosidae	*Lycosa pseudoannulata*	
Lycosidae	*Trochosa terricola*	
Salticidae	*Telamonia dimidiate*	13.33
Salticidae	*Thyene imperalis*	

Table 2. Dominance of spider species recorded in *kharif* maize crop

Family	Scientific name	% Dominance (Family)
Araneidae	*Gea theridiodes*	20
Araneidae	*Neoscona mukerjei*	
Araneidae	*Neoscona nautica*	
Araneidae	*Neoscona theisi*	
Oxyopidae	*Oxyopes birmanicus*	20
Oxyopidae	*Oxyopes heterophthalamus*	
Oxyopidae	*Oxyopes javanus*	
Oxyopidae	*Peucetia viridians*	
Lycosidae	*Pardosa Agricola*	40
Lycosidae	*Pardosa amenata*	
Lycosidae	*Pardosa milvinia*	
Lycosidae	*Pardosa Modica*	
Lycosidae	*Pardosa pseudoannulata*	
Lycosidae	*Pardosa pullata*	
Lycosidae	*Lycosa pseudoannulata*	
Lycosidae	*Pirata piraticus*	
Salticidae	*Patu digua*	10
Salticidae	*Thyene imperalis*	
Phylodromidae	*Philodromous possibleprataria*e	5
Pisauridae	*Dolomedes tenebrosus*	5

Table 3. Mean number of spiders recorded in spring maize crop

Scientific name	March		April		May		June		Total population (mean)
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	
Pardosa amenata	0.93	1.53	6.2	8.2	10.06	13	9.66	6.93	56.51[g]
Lycosa pseudoannulata	0.26	1.46	3.4	6.33	8.13	10.4	8.13	4.46	42.57[f]
Pardosa pseudoannulata	0.2	0.93	2.33	3.4	6.2	5.13	6.06	4.13	28.38[e]
Pardosa milvinia	0.13	1.4	1.8	3.3	5.8	4.53	2.2	0.8	19.96[d]
Pardosa uintana	0.06	1.2	1.86	2.73	3.33	6	3.06	1	19.24[d]
Pardosa prativaga	0.2	1.06	2.86	5.26	7.13	1.6	0.73	0.26	19.1[d]
Pardosa monticola	0	0.6	1.4	3.53	3.4	3.4	2.86	0.93	16.12[c]
Oxyopes javanus	0	0.66	1.8	3.66	4.2	3	1.2	0.8	15.32[c]
Oxyopes birmanica	0	0.33	2.46	1.6	1.8	2.53	3.73	1.73	14.18[c]
Thyene imperalis	0	0.46	1.13	1.6	1.86	0.53	0.66	0.2	6.44[b]
Leucage argyra	0	0.06	1.06	2.06	0.66	0.33	0.4	0.06	4.63[a]
Telamonia dimidiate	0	0.13	0.33	1.26	1.33	0.4	0.06	0.06	3.57[a]
Metepeiro labyrinthea	0	0	0	1.13	0.53	0.33	0	0	1.99[a]
Trochosa terricola	0	0	0.13	0.33	0.4	0.46	0.26	0.06	1.64[a]
Tetragnatha versicolor	0	0	0	0.2	0.73	0.2	0.26	0.06	1.45[a]
Total	0.12	0.65	1.78	2.97	3.70	3.45	2.62	1.43	16.74
Species richness					15				
Species evenness					0.84				
Shannon diversity index					2.29				
Simpson index					0.125				

Pardosa modica, Pardosa pullata, Patu digua, Dolomedes tenebrosus, Neoscona theisi, Oxyopes heterophthalamus, Philodromous possibleprataríae, Thyene imperalis, N. mukerjei, Neoscona nautica, Peucetia viridians, and *Gea theridiodes* were only from *kharif* crop.

Siliwal et al. (2003) observed the diversity of spiders in the temperate maize ecosystem of Kashmir and recorded 13 families, 37 species, and 28 genera. Most spiders belonged to families Lycosidae, Theridiidae, Tetragnathidae, and Salticidae. Ekka et al. (2015) recorded 118 species of spiders in the maize crop of district Raigarh, Chhattisgarh which belonged to 52 genera under 17 families out of which seven families were dominant such as Araneidae (26 species), Oxyopidae (10 species), Gnaphosidae (18 species), Thomisidae (22 species), and Lycosidae (14 species).Investigations done by Saranya et al. (2018) on the diversity of spiders in the maize ecosystem throughout the crop growth (seedling to maturity stage) in Tamil Nadu Agricultural University recorded 2,821 spiders belonging to 16 species, 10 genera and 6 families. The most abundant families recorded were Lycosidae (1671 individuals) followed by Salticidae (459 individuals) and Oxyopidae (352 individuals). The dominant species of spiders in the maize ecosystem included *Lycosa barnesi, L. pseudoannulata, Pardosa birmanica, Salticus* sp. and *Hippasa lycosina*.Tiwari and Singh (2021) recorded 29 species of spiders under 11 genera from 18 states and 3 union territories out of which 12 species were endemic. Kacar (2015) observed 212 spider species from maize cropsin Turkey of 21 genera and 18 families with Philodromidae, Salticidae, and Thomisidae as predominant and *Cyclosa algerica* as first record in spider fauna from maize crop. In Punjab, surveys of 21 fruit crops revealed the presence of 43 spider species, belonging to 23 genera and 13 families with Salticidae, Araneidae and Oxyopidae exhibiting high diversity with Araneidae (35%) as predominant (Singh et al., 2020).

CONCLUSION

Spring and kharif maize crops have abundant spider diversity especially of family Lycosidae and genus *Pardosa* during May and August months. Hence, pest management strategies, especially the use of pesticides should be used with caution during these times.

Table 4. Mean number of spiders recorded in *kharif* maize crop

Scientific name	March		April		May		June		Total population (mean)
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	
Lycosa pseudoannulata	0	1.46	4.13	7.06	8.53	10.06	8.46	4.8	44.5[e]
Pardosa milvinia	0	1.26	3.4	6.66	7.4	9.5	7.86	4.80	40.88[e]
Pardosa pseudoannulata	0	1.06	2.73	3.6	6.33	5.53	6.2	4.4	29.85[d]
Pirata piraticus	0	1.06	1.93	2.9	4.8	5.93	3.2	1.2	21.05[d]
Pardosa amenata	0	1.46	1.86	3.33	5.8	4.75	2.33	0.86	20.39[d]
Pardosa agricola	0	1.2	3	5.26	7.26	1.66	0.8	0.26	19.44[d]
Pardosa modica	0	0.73	1.46	3.73	3.5	3.46	2.33	1.06	17.27[c]
Pardosa pullata	0	0.86	1.86	3.66	4.33	3.13	1.2	1.06	16.1[c]
Patu digua	0	0.33	2	1.53	1.93	2.73	3.86	1.86	14.24[c]
Dolomedes tenebrosus	0	0.46	1.06	1.8	1.86	1.26	0.73	0.26	7.43[b]
Neoscona thewasi	0	0.33	1	0.93	1.06	0.86	0.13	0.06	5.37[b]
Oxyopes birmanica	0	0	1.13	2.2	0.73	0.4	0.33	0.13	4.92[a]
Oxyopes javanus	0	0.13	0.73	1.33	1.2	1	0.26	0	4.65[a]
Oxyopes heteropthalamus	0	0.2	0.6	0.73	0.33	0.2	0.13	0.13	2.32[a]
Philodromous possiblepratariae	0	0.06	0.33	0.6	0.73	0.26	0.26	0.06	2.3[a]
Thyene imperalis	0	0	0	1.13	0.66	0.4	0	0	2.19[a]
Neoscona mukerjei	0	0	0.2	0.26	0.53	0.46	0.26	0.06	1.77[a]
Peucitia viridans	0	0.2	0.26	0.46	0.46	0.13	0.06	0	1.57[a]
Gea theridiodes	0	0	0	0.26	0.6	0.13	0.06	0.06	1.1[a]
Neoscona nautica	0	0	0.06	0.26	0.46	0.2	0.06	0.06	1.1[a]
Total	0	0.54	1.38	2.38	2.92	2.60	1.92	1.05	12.92
Species richness					20				
Species evenness					0.81				
Shannon diversity index					2.411				
Simpson index					0.114				

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Farmers Perception and Knowledge for Management Practices of Fall Armyworm (*Spodoptera frugiperda* Smith) in Lower Shivalik Foothills of Punjab, India

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Abstract: This study aimed to gather information about farmers perception, knowledge and management practices of the introduced insect pest, the fall armyworm *Spodoptera frugiperda* (FAW) A total of 100 smallholder farmers with experience in maize cultivation were surveyed using questionnaire from village Bhattlan and Bhambowal. Most farmers use hybrid varieties (67%), while others rely on local maize varieties (33%). Very few farmers were unable to morphologically identify fall armyworm (FAW) (22%). Most farmers have experienced FAW damage in their farms (97%). Maize is mostly planted in June (when rain starts) by 94% of farmers and the highest infestation period is believed to be between July to August when crop is young and most preferred by FAW. Among those using chemical insecticides to fight FAW, their primary source of insecticides is dealers' shops (85.5%). The majority of farmers spray by themselves (81%). Only 24% farmers reported that they use the recommended dose of insecticides for management of fall armyworm in maize. Although farmers are aware of the presence of FAW in maize fields, the majority of them are unaware about the recommended insecticides and spray technology, which probably affects their ability to control the pest. Awareness campaigns addressing the issues of identification and control of the pest should be implemented targeting smallholder farmers.

Keywords: Fall armyworm, Perception, Sub-mountainous, Survey

Maize (*Zea mays* L) being one of the versatile emerging crop with wider adaptability under different agro-climatic environmental conditions (Manjanagouda and Kalyanamurthy 2018). Maize is a used in the form of food, fodder, feed and industrial raw material. Globally, around 1147.7 million metric tonnes of maize is produced from 193.7 million hectare with an average yield of 5.75 tonnes per hectare in 170 countries (Meena and Nirupma 2021). In Punjab maize occupied 93.3 thousand hectares, with a production of 410 thousand tonnes with average yield was 43.93 quintal per hectare during 2022-23 (Anonymous 2024). Insect pests are amongst the major biotic constraints causing losses in quantities and qualities in the maize crop (Singh and Singh, 2018).Among different insect pest fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) is a noctuid moth that originates from tropical and sub-tropical America (Agboyi et al., 2020). It is a polyphagous pest, known to be a dominant feeder of maize and other cereals (Montezano et al., 2018). This pest has been reported in 100 countries across the globe including India (Baloch et al., 2020). In India, fall armyworm, *Spodoptera frugiperda* (FAW) was first reported from Karnataka in 2018 and in2019 from Punjab (Sharanbassapa et al., 2019, Jitendra et al., 2019, Kerketta et al., 2020). In general, the maize infestation by FAW ranged from 26.4 to 55.9 per cent and impacted yield of 11.57 per cent (Baudron et al., 2019, Balla et al., 2019, Prasanna et al., 2018). Crop losses due to insect pests may be prevented, or reduced, by deploying effective crop protection measures, which to a large extent depend on farmers' knowledge and behaviour towards pest management (Kansiime et al., 2019, Kumela et al., 2018). In cases of economically important and invasive insect pests such as FAW, it is crucial to know how familiar farmers are with the pest, what options they have to control it and what are their main constraints and proper identification of natural enemies which are prevailing in their area. Surveys designed to ascertain farmers' knowledge and practices regarding pest management are important for pest control (Kansiime et al., 2019). Keeping these points in mind, the present study was aimed to assess perception, the knowledge, and management practices of FAW among farmers of sub-mountainous area of Punjab, India.

MATERIAL AND METHODS

This study was carried out in two villages Bhambowal (31°45'27.4"N, 75°47'51.8"E) and Bhattlan (31°56'24.0"N, 75°07'48.0"E) of district Hoshiapur Punjab India. In these areas maize is the main *kharif* crop and is cultivated in rainy season. The rainy season starts from June to July. Maize is often grown in small plots (less than 1 ha), in different cropping systems. In general, very less fertilizers or pesticides are used for the production of maize by smallholders. The survey was conducted from June to October during 2023. At the beginning of the survey, farmers were informed of the aim of the study. The survey was conducted among 100 farmers (50 in each village). Data of both villages were pooled. Information related to farmers' socioeconomic characteristics (age, gender, education level, monthly income, number of farms and land possession per household), knowledge and perceptions about FAW (morphological identification, recognition of attack symptoms, incidence and spread of the pest), management practices (methods of control, handling of insecticides) and constraints for its control were collected. Data were summarized per village. For each question, similar answers were grouped and the percentage of farmers who gave similar responses was determined for each village. Whenever two or more responses were given to the same question, they were again grouped by similarity and the percentage of farmers who gave a similar response was determined for each village. The two or more responses were given to the same question, were grouped by similarity and the percentage of farmers with similar response was determined for each district. In some cases, the percentage of farmers was determined based on the total number of farmers who gave a particular response (Canico et al., 2021).

RESULTS AND DISCUSSION

This study aimed to gather information about farmers perception, knowledge and management practices of the introduced insect pest, the fall armyworm *Spodoptera frugiperda*.

Socio-economic characteristics: The socio-economic characteristics are given in Table 1.

Cropping systems, maize varieties, purpose of production, recognition of FAW attack: Most farmers (94%) have more than 10 years of experience in maize cultivation in both villages. The primary source of maize seeds was from pesticide dealers (90.75%) and most farmers use hybrid varieties (67.The pooled data of Bhambowal and Bhattlan, depicted 92.5 of farmers plant maize as monocrop while remaining 7.5% prefer intercropping of maize with other crops.. The majority of farmers in these villages reported that they produce maize for both home consumption and sale (88%) (Table 2). About 99.5% of farmers in these villages reported incidence of FAW, but very few of them are unable to distinguish FAW larvae from other lepidopteran larvae (22%). The maize is mainly planted in June which coincides with the beginning of the rainy season. The incidence of FAW is reported to be high between July to August as in this period, maize plants are still young. Most farmers apply chemical insecticides (90%) and 10% farmers apply any other method of control of FAW like pheromone traps etc (Table 2).

Management and application of insecticides: Among those using chemical insecticides to control FAW, their primary source of insecticides is authorized dealers' (85.5%). But in some cases, insecticides are acquired by Agriculture/Horticulture Department (14.5%). The majority of farmers in these villages spray the insecticide in their fields by themselves (81%) and very few hire labour for spray (7%). Mostly farmer spray insecticide insecticides in their field but they don't wear protective clothing (90.25%) .Only 24% of the farmers reported that they use the recommended dose of insecticides while majority of farmers either use higher (40%) or lower (26.25%) dose of insecticide. The farmers using chemical insecticides usually mix two or more insecticides (70%) All farmers use backpack sprayers when applying insecticides. The common spraying intervals used by farmers are fourteen days and twenty one days. About 67% of farmers applying insecticides reported that the insecticides used are not efficient in the control of FAW. Despite the reported use of insecticides, farmers do not have training in pesticides use for management (34.50%). Although the number of sprays can go up to 7 sprays per crop cycle, but farmers generally spray 4-5 per crop cycle (93%). In the average number of sprays per crop cycle is 4.5. Most farmers reported appearance of pest as the basis for deciding to

Table 1. Socio-economic characteristics of farmers

Characteristics of respondents	Number of responses
	Bhambowal & Bhattlan (n = 100)
Gender	
Women	27
Men	73
Age/gender (years)	
Women	45
Men	47.50
Number of individuals/household	4.25
Education level	
No education	13.5
Primary education	50
Secondary education	27
Graduation	9.5
Another occupation excluding Agriculture	
Yes	41
No	59
Land possession/household (hectare)	0.78

apply insecticides (45%) but very few (27%) apply insecticide as per recommendations (Table 3).

The educational background of farmers seems to play a major role in their ability to get al.,ternative/additional jobs. Int his study no relationship was established between the level of education and knowledge of FAW, Abtew et al. (2016), pointed out the importance of education in farmers' level of knowledge of agricultural pests

Toepfer et al. (2019) reported that invasive alien species represent a serious challenge in the context of pest management because farmers and local agricultural extension workers rarely know about the presence of a newly arrived and spreading species until more damage occurs this is line

Table 2. Farmers' experience in seed procurement, maize cultivation and FAW incidence

Characteristics of respondents	Number of responses
	Bhambowal & Bhattlan (n = 100)
Seed provenience	
Authorized dealer	90.75
Own seed (previous season)	5
Neighboring farmer	1.75
Agric Department 2.5	
Experience in maize cultivation	
Between 5 and 10 years	6
More than 10 years	94.0
Type of maize variety	
Hybrid	67
Local/recommended	33
Cropping pattern	
Monocrop	92.5
Intercrop	7.5
Purpose of production	
Home consumption	5.5
Sale	6.5
Both	88
Observation of FAW larvae	
Yes	99.5
No	0.5
Ability to identify FAW larvae morphologically	
Can identify	78
Month of Sowing of maize	
Normal sowing (June)	94
July	6
Maximum Incidence of FAW	
July-August	100
Methods of control of FAW	
Chemical method	90
Using non chemical methods (pheromone traps etc.)	10

Table 3. Use of insecticides among farmers

Characteristics of respondents	Number of responses
	Bhambowal & Bhattlan (n = 100)
Source of insecticides	
Authorized dealer	85.5
Agriculture department	14.5
Spraying	
Farmer himself	81
Another family member	12
Someone hired	7
Use of protective equipment	
Partially equipped	9.75
Without any equipment	90.25
Dose of application of insecticides	
Recommended	24
Increased	40
Reduced	26.25
Unknown	9.75
Mixture of insecticides	
Mix	70
Application equipment	
Spraying interval	
7 days	14.50
14 days	48.50
21 days	28.50
30 days	8.50
Training in the handling of insecticides	
Trained	65.50
Non-trained	34.50
Application of insecticides based on	
On appearance of pest	45.0
Recommendation	27
Observation of neighboring farmers	28
Efficiency of insecticides	
Efficient	33
Not efficient (low quality)	67
Number of sprays of insecticide for FAW management in crop season	
1-3	3
4-5	93
6-7	4

with present studies where FAW an invasive pest caused high damage until farmers were aware about its biology or behaviour.

The infestation of FAW in maize fields is reported to be high between July and August. This can be explained by the fact that in this interval, maize planted in June is still in the vegetative stage which is the most preferred by FAW. Despite recommendation from the Agricultural Uniiversity to use a select range of insecticides to fight FAW (Cheema et al., 2021), only about 24% farmers in survey apply insecticides as per recommendation.

Concerning the specific case of FAW, several methods of control of the pest are reported in many countries where farmers use chemical, cultural and biological control (Kansiime et al.,. 2019), Ethiopia and Kenya where among other methods, farmers use physical and traditional methods (Kumela et al.,. 2018). It was found that cultivation of tolerant genotypes, adjusting sowing windows, and practicing specific intercultural and cropping systems measures in addition to chemical and non-chemical pest management strategies showed encouraging results for sustainable management of fall armyworm, which could protect the crop (Kumar et al., 2022). Many natural enemies have been identified in Punjab ecosystem against this pest (Bhargav et al., 2022). In this study, in contrast to other countries, chemical control was the only method used by a limited number of smallholder farmers. Further farmers are not properly using spray technology for its management. Sustainable strategies to manage this voracious insect can be in the form of cultural practices, mechanical control, botanical and insecticide applications, biological control, and host plant resistance. Biopesticides like Bt sprays, insecticides such as chlorantraniliprole, emamectin benzoate and spinetoram, and mixed cropping can be integrated for the effective management of this pest (Jindal et al., 2022). In a study conducted in Punjab during 2020, native parasitoids of *Chilo partellus* such as *Campoletis* spp. and *Chelonus formosanus* have been recorded on fall armyworm as well (Jindal et al., 2021) Furthermore, when biopesticides are combined with good crop management, they can keep pest levels under control (Bateman et al., 2018. Jindal et al., 2022). Surveys designed to ascertain farmers' knowledge and practices regarding pest management are important because they can highlight the need for the training of farmers in the identification of pests and the debunking of pest management misconceptions (Arshad et al., 2009). Understanding these factors is critically important for setting a research agenda, designing extension strategies, and formulating research that meets farmers' demands

CONCLUSIONS

Although farmers are aware of the presence of FAW in their maize fields and the majority of them are able to morphologically distinguish FAW from other caterpillars but spraying of unrecommended insecticides probably affects their ability to control the pest. Nevertheless, most farmers believe that the incidence of FAW is spreading but they are not able to manage this pest despite giving 4 to 5 sprays of insecticides. Farmers are not trained in spraying insecticides for management of fall armyworm and not spraying the recommended insecticide at proper dose and right time rather they mix the insecticides for getting rapid control. There is need to educate smallholder farmers regarding use of recommended insecticide and method of insecticide spray, time of spray, other method for management etc. effective management of this pest.

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Seasonal Incidence of Thrips *(Megalurothrips usitatus)* and Pod Bug *(Clavigralla gibbosa)* on *Kharif* Mungbean in Tropical Zone

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***Abstract*:** A two-year study (2022-2023) investigated the seasonal incidence of thrips (*Megalurothrips usitatus*) and pod bug (*Clavigralla gibbosa*) on *Kharif* mungbean in relation to weather parameters. The maximum thrips population during 2022 was observed in early, timely and late sown crops, with 2.60, 2.00 and 2.60 adults/10 flowers in 36th and 37th standard meteorological weeks (SMW), respectively. In 2023, peak thrips populations were 2.40, 0.60, and 1.32 adults/10 flowers for early, timely, and late sown crops during the 37th SMW. Correlation studies revealed a significant positive association of *M. usitatus* with temperature parameters in 2022 and with relative humidity and rainfall in 2023. The highest *C. gibbosa* populations in 2022 were 5.20, 3.50, and 4.26 adults/plant during the 37th SMW across early, timely, and late sown crops. In 2023, *C. gibbosa* first appeared in the early sown crop during the 33rd SMW (0.42 adults/plant), with peak at 3.80 adults/plant in the 38th SMW. Timely and late sown crops recorded peak *C. gibbosa* during the 37th SMW (2.76 and 3.34 adults/plant).

***Keywords*:** Correlation, *Clavigralla gibbosa,* Seasonal incidence, Mungbean, *Megalurothrips usitatus*

Mungbean (*Vigna radiata*), is one of India's most important pulse crops after chickpea and pigeon pea (Ved et al 2008). This crop is predominantly cultivated during the kharif season in the arid and semi-arid regions of India. During 2022-23 in Punjab, mungbean was grown over an area of 3.8 thousand hectares, with a total production of 3.0 thousand tonnes and an average yield of 7.80 quintals per hectare (Anonymous 2023).Despite its significance, mungbean production is constrained by numerous insect pests that cause severe yield losses. The major pests affecting mungbean include jassid (*Empoasca motti*), thrips (*Caliothrips indicus*), whitefly (*Bemisia tabaci*), semilooper (*Plusia orichalcea*), green bug (*Nezara viridula*), stem fly (*Ophiomyia phaseoli*), pod borers (*Helicoverpa armigera* and *Maruca testulalis*), tortricid moth (*Cydia ptychora*), galerucid beetle (*Madurasia obscurella*), and cutworm (*Agrotis ipsilon*) (Kumar et al., 2004 and Nitharwal and Kumawat 2013). These pests act as significant limiting factors in mungbean production, necessitating studies to understand their seasonal incidence and correlation with environmental factors. Main objective of this study to check the seasonal abundance of sucking insect pests on *kharif* mungbean.

MATERIAL AND METHODS

Mungbean variety, ML 1808 was sown at Entomological Research Farm, PAU, Ludhiana, during *Kharif* seasons of 2022-23. The crop was raised as per the PAU Package of Practices (Anonymous 2023). The seed was treated with rhizobium culture before sowing. There were three dates of sowing, 2 July (early sowing), 17 July (timely sowing), and 1 August (late sowing). The crop was kept free from insecticide spray. The crop was sown in plots measuring 5m x 10 m with spacing of 30 cm row to row and 10 cm plant to plant in randomized block design with five replications. Observations were recorded from initial occurrence of insect pests up to crop harvesting stage. The population of pod bug was recorded per plant and thrips from 10 flowers during flowering stage of crop at weekly interval during morning hours between 6:00 am to 8:00 am. Meteorological data regarding temperature (maximum and minimum), relative humidity and total rainfall were obtained from the Department of Climate Change and Agricultural Meteorology, PAU Ludhiana.

RESULTS AND DISCUSSION

In the early sown crop of 2022, the thrips (*Megalurothrips usitatus*) population gradually increased, peaking at 2.60 adults per ten flowers during the 36th SMW. The declining trend was observed from the 39th SMW, with the population reducing to 1.22 adults per ten flowers of mungbean. Similarly, in the early sown crop of 2023, the population peaked at 2.40 adults per ten flowers during the 37th SMW. Theis highlight the flowering stage as a critical period for thrips infestation, indicating that the reproductive phase of mungbean is particularly vulnerable. There was significant positive relationship between the thrips population and maximum temperature, minimum temperature, and mean temperature during 2022. In 2023, significant correlations were observed with relative humidity and rainfall (Table 1). Under timely sowing conditions, the peak thrips population during 2022 was 2.00 adults per ten flowers in the 36th SMW, whereas in 2023, was 0.60 adults per ten flowers during the 37th SMW. In the late sown crop of 2022.Tthe adult population of *M. usitatus* first appeared during the 33rd SMW (1.40 adults per ten flowers), peaking at 2.60 adults per ten flowers in the 37th SMW. During 2023, the late sown crop recorded a peak of 1.32 adults per ten flowers in the 37th SMW. Mahipal et al. (2017), also reported peak thrips populations on cowpea during the first week of October (40th SW) and a gradual buildup from the fifth week of September (39th SW). Correlation studies for 2022 showed a positive and significant relationship between M. usitatus populations and maximum temperature, minimum temperature and mean temperature. In 2023, correlations were significant with maximum temperature, minimum temperature, mean temperature and rainfall. That abiotic factors contributed to 0.29 to 0.39 per cent (R^2) and 0.11 to 0.32 per cent (R^2) of the variability in the thrips population during the *Kharif* seasons of 2022 and 2023, respectively (Table 1, 2). In 2022, early sown crop adults population of *C. gibbosa* was first observed during 33th standard meteorological week (SMW) with population of 0.62 adults/ plant and increased gradually with a peak population of 5.20 adults/ plant during 37th SMW. During 2023 early sown crop the population of *C. gibbosa* was maximum 3.80 adults/ plant during 38th SMW. The population of *C. gibbosa* exhibited a positive but non-significant correlation with maximum temperature, minimum temperature, mean temperature and relative humidity. However, in 2023, the population showed positive and significant correlation with rainfall and with RH. The population of *Clavigralla gibbosa* was monitored during timely and late sown cowpea crops in 2022 and 2023. Under timely sowing, peak populations of 3.50 and 2.76 adults/plant were observed during the 37th SMW in 2022 and 2023, respectively. Correlation analysis revealed a positive but non-significant relationship between *C. gibbosa* population and maximum, minimum, and mean temperatures, as well as relative humidity during 2022. In 2023, a significant positive correlation was observed with rainfall (r = 0.326). For late-

Table 1. Correlation of population of Thrips, *Megalurothrips usitatus* with various weather parameters (2022 and 2023)

Weather parameters	2022			2023		
	Early sowing	Timely sowing	Late sowing	Early sowing	Timely sowing	Late sowing
Maximum temperature (°C)	0.452 (<.0001)***	0.481 (<.0001)***	0.527 (<.0001)***	0.195 (0.082)	0.142 (0.207)	0.320 (0.003)**
Minimum temperature (°C)	0.363 (0.0009)***	0.287 (0.009)**	0.330 (0.002)**	0.147 (0.191)	0.108 (0.338)	0.220 (0.049)*
Mean temperature (°C)	0.408 (0.0002)***	0.364 (0.0009)***	0.409 (0.0002)***	0.172 (0.125)	0.126 (0.262)	0.266 (0.016)*
R.H. (per cent)	0.095 (0.397)	-0.146 (0.194)	-0.064 (0.567)	0.223 (0.046)*	0.158 (0.159)	0.168 (0.136)
Rainfall (mm)	-0.139 (0.218)	-0.216 (0.054)	-0.202 (0.071)	0.233 (0.037)*	0.078 (0.486)	0.403 (0.0002)***

*Significant at 0.05 level (two- tailed), **Significant at 0.01 level (two-tailed), ***Significant at 0.001 level (two- tailed)

Table 2. Correlation of population of pod bug, *Clavigralla gibbosa* with various weather parameters (2022 and 2023)

Weather parameters	2022			2023		
	Early sowing	Timely sowing	Late sowing	Early sowing	Timely sowing	Late sowing
Maximum temperature (°C)	0.101 (0.370)	0.155 (0.167)	0.092 (0.414)	-0.004 (0.966)	0.059 (0.601)	-0.012 (0.913)
Minimum temperature (°C)	0.020 (0.859)	0.068 (0.544)	-0.038 (0.731)	-0.022 (0.843)	0.003 (0.977)	-0.150 (0.181)
Mean temperature (°C)	0.039 (0.727)	0.093 (0.407)	-0.007 (0.947)	-0.013 (0.905)	0.025 (0.823)	0.108 (0.340)
R.H. (per cent)	0.049 (0.665)	0.089 (0.428)	-0.008 (0.937)	0.238 (0.033)*	0.164 (0.145)	0.059 (0.600)
Rainfall (mm)	-0.133 (0.238)	-0.149 (0.184)	-0.177 (0.115)	0.339 (0.0021)**	0.326 (0.003)**	0.329 (0.002)**

*Significant at 0.05 level (two- tailed), **Significant at 0.01 level (two-tailed), ***Significant at 0.001 level (two- tailed)

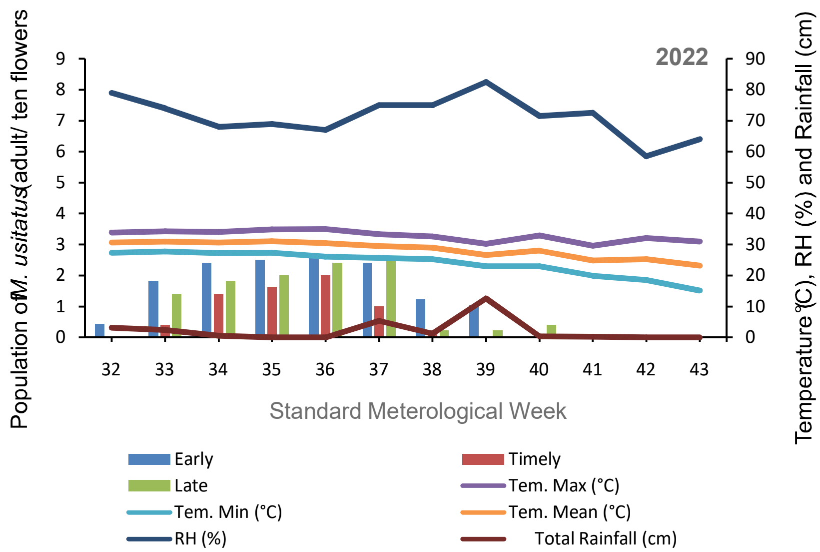

Fig. 1. Seasonal incidence of Thrips, *Megalurothrips usitatus* on *Kharif* mungbean (2022)

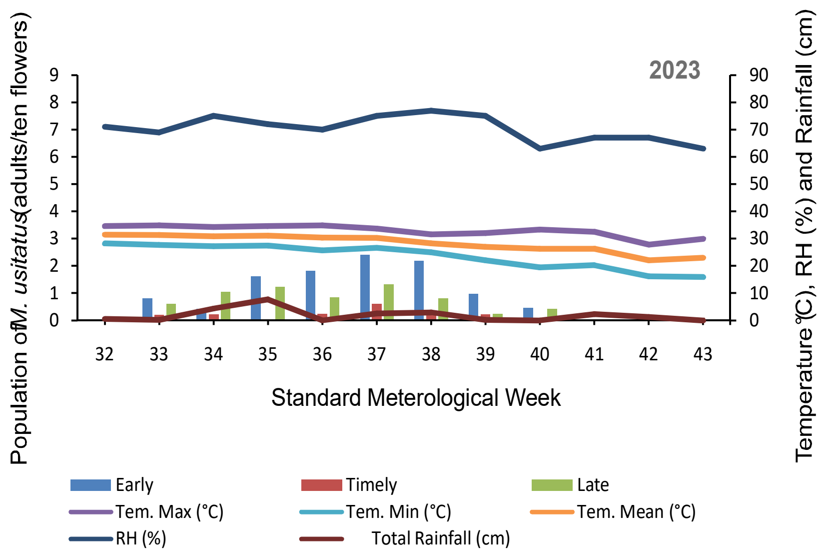

Fig. 2. Seasonal incidence of Thrips, *Megalurothrips usitatus* on *Kharif* mungbean (2023)

Table 3. Multiple regression equation of thrips and pod bug population with various environmental factors on different dates of sowing during 2022 and 2023

Date of sowing	Insect	Year	Multiple regression equation	Coefficient of determination R^2
Early sowing	Thrips	2022	$Y= -12.43+9.03X_1+8.60X_2-17.32X_3+0.03X_4-0.005X_5$	0.39
	Thrips	2023	$Y= -15.20-5.31X_1-5.81X_2+11.28X_3+0.12X_4+0.007X_5$	0.21
	Pod bug	2022	$Y= -11.4+18.75X_1+18.17X_2-36.80X_3+0.09X_4-0.006X_5$	0.46
	Pod bug	2023	$Y= -26.98-9.17X_1-10.06X_2+19.38X_3+0.27X_4+0.01X_5$	0.41
Timely sowing	Thrips	2022	$Y= -5.75+3.45X_1+3.22X_2-6.45X_3-0.01X_4-0.002X_5$	0.29
	Thrips	2023	$Y= -3.43-1.37X_1-1.48X_2+2.89X_3+0.03X_4-0.0008X_5$	0.11
	Pod bug	2022	$Y= -11.07+11.91X_1+11.40X_2-23.17X_3+0.08X_4-0.004X_5$	0.44
	Pod bug	2023	$Y= -15.61-5.59X_1-6.15X_2+11.87X_3+0.14X_4+0.008X_5$	0.30
Late sowing	Thrips	2022	$Y= -11.88+6.43X_1+5.97X_2-12.07X_3+0.01X_4-0.003X_5$	0.37
	Thrips	2023	$Y= -6.68-3.65X_1-3.91X_2+7.68X_3+0.03X_4+0.01X_5$	0.32
	Pod bug	2022	$Y= -10.42+16.95X_1+16.39X_2-33.22X_3+0.08X_4-0.006X_5$	0.48
	Pod bug	2023	$Y= -29.11-6.22X_1-7.37X_2+13.83X_3+0.25X_4+0.01X_5$	0.41

X_1=Max. Temp, X_2=Min. Temp, X_3=Mean Temp, X_4= RH, X_5=Rainfall

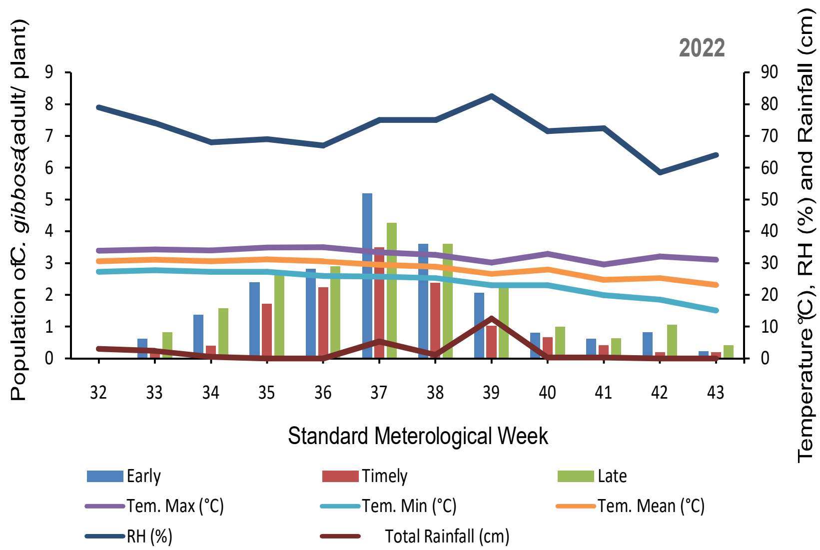

Fig. 3. Seasonal incidence of pod bug, *Clavigralla gibbosa* on *Kharif* mungbean (2022)

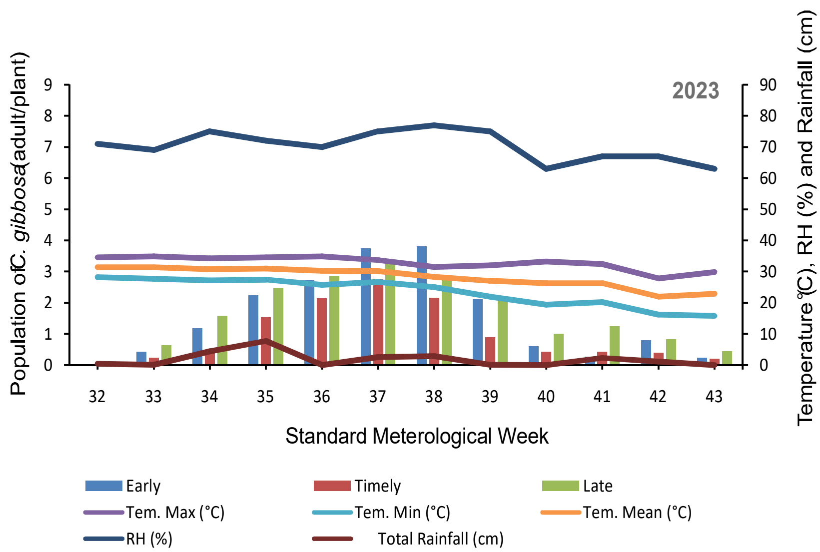

Fig. 4. Seasonal incidence of pod bug, *Clavigralla gibbosa* on *Kharif* mungbean (2023)

sown crops, peak populations of 4.26 and 3.34 adults/plant occurred during the 37th SMW in 2022 and 2023, respectively. Correlation analysis indicated a negative, non-significant relationship with minimum temperature, mean temperature, relative humidity, and rainfall in 2022, whereas in 2023, a positive but non-significant correlation was observed with relative humidity and mean temperature. The findings align partially with earlier studies. Singh et al. (2002) reported a positive correlation of *C. gibbosa* with maximum and minimum temperatures but an inverse relationship with relative humidity in cowpea crops. Kumar and Nath (2004) found that rainfall and high relative humidity favored *C. gibbosa* development in pigeonpea, while Nayak et al. (2004) recorded negative correlation of *Clavigralla* spp. with minimum temperature and relative humidity in black gram. Regression analysis demonstrated that weather factors explained 0.44-0.48% (R^2) and 0.30-0.41% (R^2) of population variation in 2022 and 2023, respectively. Contrastingly, Yadav and Singh (2013) emphasized the significance of crop developmental stages over climatic factors, suggesting that plant age plays a critical role in determining pod bug infestation levels.

CONCLUSION

The early sown crop consistently recorded higher thrips populations compared to timely and late sown crops. That weather parameters, particularly temperature, relative humidity, and rainfall, had varying degrees of influence on thrips dynamics across different sowing conditions and years. The significant positive correlations with maximum temperature in 2022 and rainfall in 2023 underscored the role of abiotic factors in shaping population trends. Correlations between pod bug populations and abiotic factors were generally weak, rainfall and relative humidity had a significant positive influence during certain periods. Integrating pest monitoring with abiotic factor analysis and crop development stages can improve pest management strategies for mungbean crops.

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Prevalence of Citrus Greening Disease and Its Integrated Management under Punjab Conditions

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Abstract: Citrus greening or Huanglongbing (HLB) is the most destructive disease limiting the quality of fruit production in India associated with *Candidatus* Liberibacter asiaticus in Asia. The disease produces typical symptoms of leaf mottling, yellowing and green islands on leaves of citrus plants. During the surveillance (2019-2021), the disease was present in various citrus growing areas of Punjab viz., Hoshiarpur, Fazilka, Faridkot and Ludhiana with incidence varied from 7.99 percent to 16.60 percent in the districts of Faridkot and Hoshiarpur, respectively. The disease index (PDI) was highest in Ludhiana and lowest in Faridkot. Disease management generally depends on the early detection of symptoms and chemical control of insect vector. Ten treatments were selected which includes the different combinations of nutrients, antibiotics, growth regulator and insecticide. Among the different treatments, zinc sulphate + manganese sulphate + boric acid, tetracycline hydrochloride and 2,4-dichlorophenoxyacetic acid (2,4-D sodium salt) was proved significantly superior to other treatments in reducing the percent disease index (13.99) and providing 59.29 percent disease control with significant increase in yield (54.87 kg/tree) as compared to control (38.32 kg/tree).

Keywords: Citrus greening, *Candidatus* Liberibacter asiaticus, Prevalence, Integrated management, Chemicals, Antibiotics

Citrus, a member of family Rutaceae, is grown worldwide including India and occupies a place of prime importance among major fruits in the country. In India, citrus is the third largest fruit crop after mango and banana with estimated production of 1,40,71 thousand MT from a cultivated area of 1064 thousand ha (Anonymous 2023). The important commercial citrus fruits in India are mandarins (*Citrus reticulata* Blanco), sweet orange (*C. sinensis* Osbeck) and acid lime (*C. aurantifolia* Swingle) (Jagtap et al., 2013). Punjab is the largest producer of citrus especially Kinnow with 47,862 ha area and production of 12,84,211 MT (Anonymous 2024). Kinnow is mostly grown in five districts of Punjab viz., Fazilka, Hoshiarpur, Mansa, Muktsar and Bathinda which contribute for 88% of total Kinnow production in Punjab. There are number of biotic and abiotic factors that are responsible for low productivity and widespread problem of citrus decline in India. Among them, Huanglongbing (HLB) or citrus greening (CG) has proven to be the most problematic due to the fastidious nature of its non-culturable Gram-negative bacteria *Candidatus* Liberibacter (Jagoueix et al., 1994). Due to the long latency period, uneven distribution of pathogen and confusing disease symptoms produced in the host, the pathosystem becomes even more complicated (Manjunath et al., 2008). Citrus greening disease can cause nutrient deficiency in citrus plants which affects the plant canopy and finally the yield of plant. Keeping in view the importance and devastating potential of the disease, the present investigation was undertaken to study the prevalence and integrated management of the disease under Punjab conditions.

MATERIAL AND METHODS

Prevalence of greening in citrus orchards of Punjab: The prevalence of citrus greening disease was observed in the citrus growing districts of Punjab namely Hoshiarpur, Fazilka, Faridkot and Ludhiana during the year 2019-2021. The surveys were conducted during the months of September-November which are considered optimum for the development of characteristic symptoms of the disease. Fifty trees of the cultivar Kinnow mandarin were randomly selected per site in each district. The canopy of the tree from each geographical direction was observed for greening symptoms. The Percent Disease Index (PDI) was recorded using 0-4 disease rating scale given by Gottwald et al., (2007) (Table 1). PDI and Percent disease incidence were calculated using the following formula:

$$\text{PDI} = \frac{\text{Sum of individual ratings}}{\text{Number of plants examined} \times \text{Maximum disease rating}} \times 100$$

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Integrated management of greening disease under field conditions: Ten treatments of different nutrients, antibiotics, growth regulators and insecticide were selected in different combinations to evaluate their efficacy in managing the disease in a Randomized Block Design (RBD) at Punjab Agricultural University, Ludhiana during the year 2020-2022 on the cultivar Kinnow (Table 2). Ten treatments were kept and each treatment was replicated thrice. The treatments, T_1 (zinc sulphate + manganese sulphate), T_2 (boric acid), T_3 (T_1+T_2) and T_6 (2,4-dichlorophenoxyacetic acid (2,4-D) sodium salt) were applied as foliar spray during the end of April and mid of August. Two sprays of treatments T_4 (tetracycline hydrochloride) and T_5 (streptocycline) were given during August-September at 45 days interval. Recommended insecticide T_9 (imidacloprid) was sprayed in each treatment during March and again in first week of September. PDI based on the visual symptoms was recorded and percent disease control was calculated. The yield of citrus trees per treatment was also recorded. Canopy volume was calculated by using the following formula:

Canopy Volume = 0.5236 × H × (N-S) × (E-W) (William 1983)

where, H= Height of tree, N-S and E-W= Spread of plant in North-South and East-West directions

RESULTS AND DISCUSSION

Disease survey: During the surveillance, the trees showing typical greening symptoms viz., leaf mottling, yellowing and green islands were rated as infected (Fig. 1). Different locations of Punjab were surveyed to record the prevalence, incidence and PDI and it was observed that the disease was widespread in different locations of Punjab (Table 3 and 4). Maximum mean disease prevalence (33.33%) was in Ludhiana district while minimum was in Faridkot (19.31%). However, number of sites in Ludhiana (9) was comparatively lesser than the two major citrus growing areas of Hoshiarpur (33) and Fazilka (36). In the current assessment, the disease was present at more than thirty percent citrus orchards i.e. 32.60 % and 33.33% at Hoshiarpur and Ludhiana, respectively.

The disease incidence and PDI were further recorded during all three years (2019 to 2021) in these four districts of Punjab (Table 4). The maximum mean disease incidence (16.60 %) was in Hoshiarpur district in each year followed by Ludhiana (15.37%) and Fazilka (9.21%) whereas; the PDI was at par in Ludhiana district (11.98%) as well as Hoshiarpur district (11.49%). The minimum disease incidence of 7.98% and PDI of 8.76% was in Faridkot.

These findings are in conformity with those of Das (2008) who observed 30-40 percent disease incidence in Malta Sweet orange and 3-15 percent in Kinnow mandarin from

Table 1. Disease score used for assessment of PDI given by Gottwald et al (2007)

Disease score	Disease rating	Description
0	No symptoms	No greening symptoms observed on the plant canopy
1	Mild	Greening symptoms up to 25% of the canopy
2	Moderate	26-50% canopy symptoms
3	Severe	51-75% canopy symptoms
4	Very severe	76-100% canopy symptoms

Table 3. Prevalence of citrus greening in different districts of Punjab on the basis of visual symptoms during 2019-2021

District	Prevalence (%)			Mean
	2019	2020	2021	
Fazilka (36)	25.00	27.77	30.00	27.59
Faridkot (30)	16.66	18.18	23.08	19.31
Hoshiarpur (33)	28.57	30.77	38.46	32.60
Ludhiana (9)	33.33	33.33	33.33	33.33
Total (108)/Mean	25.89	27.51	31.22	28.21

Figure in parenthesis include number of sites visited in each district

Table 2. Chemicals evaluated for management of citrus greening disease

Treatment	Description	Time of application
T_1	Zinc sulphate (4.7 g/litre) + Manganese sulphate (3.3g/litre)	April and mid August
T_2	Boric acid (0.05 g/litre)	April and mid August
T_3	T_1 + T_2	April and mid August
T_4	Tetracycline hydrochloride (0.6g/litre)	August-September
T_5	Streptocycline (0.025g/litre)	August-September
T_6	2,4-Dichlorophenoxyacetic acid (2,4-D sodium salt) (0.01g/litre)	April and mid August
T_7	T3+T4+T6	April and mid; August August-September
T_8	T3+T5+T6	April and mid August; August-September
T_9	Imidacloprid (17.8 SL) (0.4ml/litre)	March and September
T_{10}	Control	-

Punjab. The incidence varied from 12.5-30 percent in Sikkim mandarin, 10-20 percent in Darjeeling mandarin, 5-8.3 percent in Khasi mandarin and 12.5 percent on Sweet orange cultivars in Northeastern region of India (Das et al., 2007). Gupta et al. (2012) observed 40 percent of disease incidence in Kinnow mandarin at Hoshiarpur district of Punjab. Ghosh et al. (2013) also observed greening like symptoms among Cleopatra, Musambi, Rangpur Lime, Acid Lime and Rough Lemon and reported incidence between 8.2-41.5 percent in Ahmednagar and 7.8-28.3 percent in Pune districts of Maharastra. Sonavane and Venkataravanappa (2017) observed the incidence of 35-64.2 percent of greening disease in Coorg mandarin in Kodagu and Hassan districts of Karnataka. Similarly, Arora (2017) reported that citrus greening was more prevalent in the Central and Sub-mountainous regions, particularly on older trees.

Integrated management of greening disease: The treatment T_7 (zinc suphate + manganese sulphate + boric acid+tetracycline hydrochloride and 2,4-

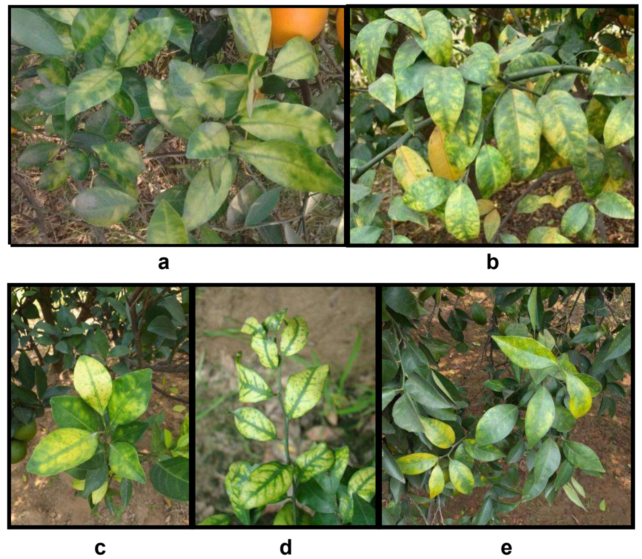

Fig. 1. Leaf mottling (a, b), green island (c, d, e) type of symptoms on citrus variety Kinnow naturally infected with citrus greening bacterium

Table 4. Disease incidence and percent disease index of citrus greening in different districts of Punjab during 2019-2021

Districts	2019		2020		2021		Mean disease incidence (%)	Mean PDI (%)
	Disease incidence (%)	PDI (%)	Disease incidence (%)	PDI (%)	Disease incidence (%)	PDI (%)		
Fazilka (36)	9.09	7.72	9.17	8.69	9.37	11.63	9.21	9.35
Faridkot (30)	7.14	7.15	8.00	8.33	8.82	10.81	7.98	8.76
Hoshiarpur (33)	15.38	9.40	16.66	12.06	17.77	13.01	16.60	11.49
Ludhiana (9)	13.33	11.07	15.83	11.29	16.66	13.58	15.27	11.98
Mean	11.24	8.84	12.41	10.09	13.15	12.26		

Figure in parenthesis include number of sites vis ited in each district

Table 5. Effect of different treatments on percent index of greening disease in Kinnow mandarin during 2020-21 at PAU, Ludhiana

Treatment	Percent disease index				Mean percent disease index		Disease control (%)
	2020		2021		Before treatment	After treatment	
	Before treatment	After treatment	Before treatment	After treatment			
T1	22.8	20.3	23.7	21.9	23.24	21.12	38.55
T2	23.1	22.1	23.9	21.0	23.50	21.53	37.36
T3	25.8	19.7	21.5	18.3	23.65	18.96	44.83
T4	24.1	22.7	24.0	21.0	24.07	21.86	36.40
T5	22.5	22.0	24.3	22.7	23.41	22.30	35.12
T6	24.6	23.2	25.0	24.2	24.79	23.67	31.13
T7	23.4	15.6	16.9	12.4	20.15	13.99	59.29
T8	25.0	18.7	19.1	16.3	22.02	17.49	49.11
T9	22.7	21.9	24.5	22.9	23.57	22.40	34.82
T10	25.6	30.5	34.0	38.9	29.80	34.37	-
CD (p=0.05)	1.27	1.78	1.24	1.70	1.26	1.74	

Table 6. Effect of different treatments on canopy volume and fruit yield in Kinnow mandarin during 2020-21 at PAU, Ludhiana

Treatment	Canopy volume (m^3)		Mean canopy volume (m^3)	Fruit yield (kg/tree)		Mean fruit yield (kg/tree)
	2020	2021		2020	2021	
T1	22.3	23.1	22.70	43.74	46.24	44.99
T2	21.3	23.8	22.55	42.48	46.10	44.29
T3	22.9	24.7	23.82	47.74	51.86	49.80
T4	21.8	22.5	22.15	43.67	45.50	44.58
T5	21.9	22.5	22.20	43.10	45.27	44.18
T6	22.0	23.5	22.75	43.36	45.62	44.49
T7	23.9	27.3	25.60	53.25	56.50	54.87
T8	23.4	25.0	24.20	51.75	55.00	53.37
T9	20.5	22.4	21.45	42.50	43.83	43.16
T10	21.2	21.0	21.10	40.37	36.28	38.32
CD (p= 0.05)	0.04	1.49	0.77	3.29	2.63	2.96

dichlorophenoxyacetic acid (2,4-D sodium salt) proved significantly superior to other treatments by reducing the percent disease index (13.99) (Table 5). The percent disease control was highest with T_7 (59.29) treatment followed by T_8 (49.11) and T_3 (44.83). The maximum canopy volume was also highest in T_7 (25.57 m^3). Foliar spray of 2,4-dichlorophenoxyacetic acid (2,4-D sodium salt) (T_6) was, however, the least effective. The plants sprayed with treatment T_7 yielded 54.87 kg/ tree followed by treatment T_8 with 53.37 kg/tree (Table 6). Zhang et al. (2011) also reported that the combination of penicillin and streptomycin (PS) was effective in suppressing the Las bacterium but due to the short term effect of antibiotics, it is not considered as sustainable approach in HLB control (Bove 2006).

Foliar spray of systemic insecticides such as imidacloprid, fenpropathrin, chlorpyrifos and dimethoate is an effective approach to manage citrus psylla (Tiwari et al., 2011). Arora et al., (2020) also reported that the best management programme for citrus greening is integrated approach including use of disease free nursery plants, management of zinc and manganese deficiency and citrus psylla vector. Integrated management approaches consisting of use of disease free nursery planting material, foliar spray of tetracycline hydrochloride, application of recommended doses of both micro and macro nutrients and foliar spray of insecticides at the time of new flush emergence can help in reducing losses due to citrus greening disease, psylla vector and prolonging lifespan of citrus (Anonymous 2022).

CONCLUSION

From survey of the citrus orchards during the three consecutive years, it was evident that the maximum greening disease incidence was in Hoshiarpur district in each year followed by Ludhiana and Fazilka. The intensity of citrus greening disease can be reduced by foliar spray of micro nutrients like zinc sulphate, manganese sulphate, boric acid, growth regulator 2,4-D sodium salt in the months of April and mid August and two sprays of tetracycline hydrochloride at an interval of 45 days during the months of August-September. Application of micronutrients reduces the nutrient deficiency symptoms caused by citrus greening disease and regain the vigor of plant whereas antibiotics slow down the progress of disease in the plant.

AUTHOR'S CONTRIBUTION

Conceptualization of research work and designing of experiments (AA, AK, MSH); Survey for disease incidence (SK, AA, AK MG); Execution of field experiments and data collection (SK, AA, AK, MSH); Analysis of data and interpretation (AA, SK, MSH, GSS); Preparation of manuscript (AA, AK, SK).

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Management of Pea Wilt Incitant by *Fusarium oxysporum* f. sp. *pisi* (Linford) using AM Fungi *Gigaspora margarita* Inoculation at various Phosphorus Amendments in Pea (*Pisum sativum* L.)

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Abstract**:** The pea (*Pisum sativum* L.) is an important vegetable and pulse crop in subtropical and temperate areas. The present study investigated the potential of *Gigaspora margarita*, an arbuscular mycorrhizal fungus (AMF), as a biocontrol agent against *Fusarium oxysporum* f. sp. *pisi* (FOP), the pathogen causing pea wilt. Experiments were conducted under pot house conditions at two locations in Punjab (Ludhiana and Abohar) during the *rabi* season of 2022-2023 to assess the impact of AMF on mycorrhization and disease suppression when co-inoculated with the pathogen.The pre-treating plants with AMF significantly reduced wilt disease incidence compared to inoculating with FOP at sowing followed by AMF application. Early AMF inoculation promoted mycorrhizal colonization, resulting in up to an 86.67% reduction in disease intensity at 40 mg phosphorus per kg of soil. This suggests that soil treatment with AMF before planting is an effective bioprotective strategy against pea wilt.

Keywords**:** Arbuscular Mycorrhizal Fungi, *Gigaspora margarita*, *Fusarium oxysporum* f. sp. *pisi*, Pea wilt

Pea (*Pisum sativum* L.) ranks as the fourth most significant cultivated legume, following common bean, cowpea, and chickpea globally (Faostat 2019). In India, after chickpeas and lentils, pea rank as the third most popular *rabi* pulse crop. Pea cultivation covers 589 thousand hectares, yielding a production of 6130 metric tons (Indiastat 2021-22). In Punjab, during 2021-22, pea was cultivated on 44.1 thousand hectares, producing approximately 469.4 thousand tons, with an average yield of 42.90 quintals per acre (Anonymous 2022).

Pea production faces significant challenges due to various diseases affecting its productivity. In Northern India, wilt is emerging as a challenge for pea cultivation, the primary cause of wilt is fungus *Fusarium oxysporum* f. sp. *pisi* (linford) Snyder and Hansen. This soil-borne fungus can persist in the soil as a chlamydospores for more than ten years (Deng et al*.,* 2022). In the Hoshiarpur district of Punjab, crop losses due to a disease complex caused by *Fusarium solani* f. sp. *pisi* and *F. oxysporum* f. sp. *pisi* were in the range between 13.9 and 95 percent (Kripalini et al., 2019).

Effective management of wilt is essential to mitigate substantial losses in pea crops. However, growers face various challenges in controlling this disease, as conventional chemical treatments provide only limited control, and resistant varieties are unavailable against this disease in Punjab. Furthermore, chemical pesticides are becoming progressively costly and less efficient due to the co-evolution and emergence of disease resistance and their unintended consequences on non-target organisms. Bioprotection, a well-documented and increasingly significant approach, is frequently used to supplement or substitute chemical methods. Arbuscular mycorrhizal fungi (AMF) have great potential as a biocontrol agent. The study examines the impact of *Fusarium oxysporum* f. sp. *pisi* on mycorrhization (root colonization and AMF spore population/100-gram soil) and effects of AMF on inhibiting disease caused by *Fusarium oxysporum* f. sp. *pisi*.

MATERIAL AND METHODS

The experiments were carried out during the 2022-23 *Rabi* season at two locations, Ludhiana and Abohar, under controlled pot house conditions using sterilized soil. The Arbuscular Mycorrhizal Fungus *Gigaspora margarita* was procured as pure culture from the Centre for Natural Biological Resources and Community Development (CNBRCD), Bengaluru, Karnataka. AMF colonization was assessed by staining the roots following the procedure outlined by Phillips and Hayman (1970). Arbuscular mycorrhizal (AM) infection was quantified following the procedure recommended by Biermann and Lindermann (1981). AMF spores were isolated using the wet sieving and decanting method (Gerdemann and Nicolson 1963). The spore population was then quantified using a counting dish in a 25 ml spore suspension. *Gigaspora margarita* inoculum was multiplied on the roots of pea plants in sterilized soil within the pot house. A thin layer of AMF inoculum weighing 10grams containing AMF spores, AMF hyphae and root bits were placed in 4 Kg of sterilized soil 4 cm below the upper surface of the soil in an earthen pot (Singh et al., 2019). "MATAR AGETA 7" pea variety was used in the present study. Pathogen *Fusarium oxysporum* f. sp. *pisi* (ITCC Number = 4814) was obtained from the Indian Type Culture Collection, IARI. FOP was maintained by periodic transfer on PDA slants and the culture was stored at 4°C for further use. The experiments aimed to evaluate the impact of arbuscular mycorrhizal fungi on the disease inhibition of pea wilt. Four treatments were applied: 1. Arbuscular mycorrhizal fungus (AMF) only, 2. AMF/Pathogen (AMF inoculation at sowing and pathogen inoculation after 5 days of sowing), 3. Pathogen/AMF (Pathogen inoculation at sowing and AMF inoculation after 5 days of sowing) and 4. Pathogen (*Fusarium oxysporum* f. sp. *pisi*) alone. The experiment also included three levels of phosphorus in the soil at 0, 40 and 80 mg phosphorus/kg soil (P_0, $P_{40,}$ P_{80}). All treatments were replicated three times in both locations. Colonization and spore population observations were recorded by uprooting the plants 20, 40, 60 and 80 days after sowing. For calculating the percentage of disease inhibition by *Gigaspora margarita*, 10 plants were maintained in pots for each treatment.

Stataistical analysis: All the data collected were subjected to statistical analysis using analysis of variance with a complete randomized design in the CPCS1 program. The necessary, data were transformed using the arcsine transformation.

RESULTS AND DISCUSSION

Effect of pathogen and phosphorus on mycorrhization

The study evaluated root colonization and spore populations of the arbuscular mycorrhizal fungus (*Gigaspora margarita*) under both the presence and absence of the pea wilt pathogen, *Fusarium oxysporum* f. sp. *pisi*.

Mycorrhizal effect:AMF *Gigaspora margarita* root colonization increased over time. When AMF alone was inoculated, colonization increased from 30.48 per cent at 20 DAS to 74.72 per cent at 80 DAS in Ludhiana (Table 1), and from 25.71 per cent at 20 DAS to 71.45 per cent at 80 DAS in Abohar (Table 2). However, the presence of the pathogen (FOP*)* negatively impacted AMF colonization. When AMF was applied at sowing and the pathogen was added 5 days later (AMF/Pathogen), colonization increased from 25.68 per cent at 20 DAS to 67.67 per cent at 80 DAS in Ludhiana, and from 20.88 per cent at 20 DAS to 61.44 per cent at 80 DAS in Abohar. This was higher than the colonization observed when the pathogen was introduced at sowing and AMF was applied 5 days later (Pathogen/AMF), which increased from 21.05 per cent at 20 DAS to 61.56 per cent at 80 DAS in Ludhiana, and 17.04 per cent at 20 DAS to 55.30 per cent at 80 DAS in Abohar. No mycorrhizal colonization was detected in the treatment with only the pathogen at either location.

Similar trends were observed for the spore population of AMF *Gigaspora margarita* in pea plant rhizospheric soil. In Ludhiana, the spore population in 100 grams of soil increased from 333.33 spores at 20 DAS to 778.67 spores at 80 DAS (Table 3), and in Abohar, it increased from 258.33 spores at 20 DAS to 660.56 spores at 80 DAS (Table 4) when AMF alone was inoculated. The presence of the pea wilt pathogen FOP, influenced spore formation by *Gigaspora margarita*. In AMF/Pathogen treatment, 100 grams of rhizosphere soil showed an average of 252.78 spores at 20 DAS, increasing to 650.67 spores in the soil at 80 DAS in Ludhiana, and in Abohar, the spore population was 186.11 spores at 20 DAS, increasing to 544.44 spores at 80 DAS. In the Pathogen/AMF treatment, the average spore counts of 100 grams rhizosphere soil were 211.11 spores at 20 DAS to 560.67 spores at 80 DAS in Ludhiana, and 136.11 spores at 20 DAS to 472.22 spores at 80 DAS in Abohar. No mycorrhizal spores were found at any stage of crop growth in the treatment with only the pathogen at both locations.

Singh et al. (2019) they also reported that root colonization by *Glomus macrocarpon* increased from 31.7 percent at 15 DAS to 72.2 percent at 60 DAS in spring season (2017) and 28.3 percent at 15 DAS to 69.3 DAS in spring season (2018) of mungbean crop. Similarly, Kaur (2021) also observed increased root colonization in chickpea with crop maturity from 42.7 percent at 30 DAS to 79.3 percent at 120 DAS in chickpea crop. Sohrabi et al (2015), noted a decline in *Glomus mosseae* colonization in chickpea roots from 78.75 per cent to 57.50 per cent in the presence of *Fusarium solani* f.sp. *pisi*.

Phosphorus effect: Phosphorus application significantly enhanced AMF colonization and spore population across all treatments in both Ludhiana and Abohar. In Ludhiana, AMF colonization for the P_{40} increased from 23.20 per cent at 20 DAS to 55.50 per cent at 80 DAS, while in P_0 treatments increased from 15.70 to 45.50 percent, and for P_{80} treatments increased from 18.99 to 51.96 per cent (Table 1). In Abohar, colonization for the P_{40} treatments increased from 19.75 per cent at 20 DAS to 52.25 per cent at 80 DAS, with the P_0 treatments increasing from 11.72 per cent to 41.38 per cent, (Table 2). Similarly, in Ludhiana, the spore population per 100 grams of rhizospheric soil for the P_{40} treatments increased from 235.41 spores at 20 DAS to 574.67 sporesat 80 DAS, while in P_0 treatments increased from 168.75 spores to

417.00 spores, and in P_{80} increased from 193.75 spores to 500.33 spores (Table 3). In Abohar, the spore population for P_{40} treatments increased from 179.17 spores at 20 DAS to 482.92 spores at 80 DAS, with the P_0 treatment increasing from 118.75 spores to 395.83 spores, and in P_{80} treatment rising from 137.50 spores to 435.42 spores (Table 4). These results indicate that phosphorus application, particularly at 40 mg phosphorus/kg soil, significantly enhances AMF colonization and spore population over time in both Ludhiana and Abohar.

Singh et al. (2017) demonstrated that phosphorus levels significantly influenced the mycorrhization of AMF *G. bagyarajii* in the presence and absence of the pathogen *F. oxysporum* f. sp. *ciceri*. Singh et al. (2019) observed that an optimal phosphorus level (40mg/kg of soil) plays a crucial role in enhancing mycorrhizal colonization and spore population of *Glomus macrocarpon* even in the presence of the dry root rot pathogen *Macrophomina phaseolina* in mungbean.

Interaction between mycorrhiza and pathogen: There was a significant interaction between mycorrhiza and the pathogen, particularly influenced by phosphorus application. Among the arbuscular mycorrhiza-inoculated (AMF) treatments, the highest root colonization was consistently observed, with the most significant enhancement occurring at 40 mg phosphorus/kg of soil (P_{40}) compared to both no phosphorus (P_0) and high phosphorus (P_{80}) levels. Specifically, in Ludhiana, AMF colonization increased from 37.31 per cent at 20 DAS to 82.17 per cent at 80 DAS, while in Abohar, it increased from 31.22 per cent at 20 DAS to 79.80 per cent at 80 DAS under P_{40} treatment conditions. The presence of the pathogen reduced AMF root colonization percentages at all three levels of phosphorus, but there was a significant increase in root colonization even in the presence

Table 1. Colonization of *Gigaspora margarita* in roots of pea plants in the presence and absence of *Fusarium oxysporum* f. sp. *pisi* at Ludhiana

Treatment	Colonization percentage after days of sowing			
	20	40	60	80
Mycorrhizal effect				
AMF	30.48 (33.49)	48.89 (44.35)	65.28 (53.88)	74.72 (59.80)
AMF/Pathogen	25.68 (30.43)	40.56 (39.53)	57.67 (49.39)	67.67 (55.33)
Pathogen/AMF	21.05 (27.29)	33.06 (35.07)	51.56 (45.87)	61.56 (51.66)
Pathogen	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD (p=0.05)	0.89	1.62	0.86	0.89
Phosphorus Effect				
P_0	15.70 (23.33)	26.25 (30.80)	40.25 (39.36)	45.50 (42.40)
P_{40}	23.20 (28.78)	35.63 (36.36)	47.66 (43.65)	55.50 (48.14)
P_{80}	18.99 (25.82)	30.00 (33.16)	42.96 (40.94)	51.96 (46.10)
CD (p=0.05)	0.98	2.04	0.49	0.46
Interaction effect between mycorrhiza and pathogen				
P_0 AMF	25.37 (30.23)	42.5 (40.67)	58.67 (49.97)	65.67 (54.11)
P_{40} AMF	37.31 (37.63)	58.33 (49.79)	72.83 (58.58)	82.17 (65.00)
P_{80} AMF	28.76 (32.33)	45.83 (42.57)	64.33 (53.32)	76.33 (60.90)
P_0 AMF/Pathogen	20.27 (26.75)	33.33 (35.24)	54.67 (47.33)	61.67 (51.74)
P_{40} AMF/Pathogen	30.94 (33.78)	47.5 (43.55)	61.67 (51.73)	72.67 (58.47)
P_{80} AMF/Pathogen	25.82 (30.52)	40.83 (39.60)	56.67 (48.81)	68.67 (55.94)
P_0 Pathogen/AMF	17.16 (24.43)	29.17 (32.67)	47.67 (43.64)	54.67 (47.66)
P_{40} Pathogen/AMF	24.57 (29.69)	36.67 (37.24)	56.17 (48.53)	67.17 (55.03)
P_{80} Pathogen/AMF	21.41 (27.54)	33.33 (35.24)	50.83 (45.46)	62.83 (52.42)
P_0 Pathogen	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
P_{40} Pathogen	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
P_{80} Pathogen	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD (p=0.05)	(1.57)	1.79	1.29	1.30

of the pathogen at P_{40}. In the treatment AMF/Pathogen and there was an increment in root colonization from 30.94 per cent at 20 DAS to 72.67 per cent at 80 DAS in Ludhiana (Table 1) and from 26.81 per cent at 20 DAS to 68.21 per cent at 80 DAS in Abohar (Table 3) under P_{40} conditions. Whereas in Pathogen/AMF treatment, the AMF colonization increased from 24.57 per cent at 20 DAS to 67.17 per cent at 80 DAS in Ludhiana and from 20.99 per cent at 20 DAS to 60.98 per cent at 80 DAS in Abohar under P_{40} conditions. The interaction effect on the spore population of AMF *Gigaspora margarita* in the 100 grams of rhizospheric soil were also significant. At the P_{40} level, the AMF alone treatment showed the highest spore population increase from 391.67 spores at 20 DAS to 864.33 spores at 80 DAS in Ludhiana (Table 2) and from 333.33 spores at 20 DAS to 756.67 spores at 80 DAS in Abohar (Table 4).

In AMF/Pathogen treatment, the spore population increased from 291.67 spores at 20 DAS to 763.33 spores at 80 DAS in Ludhiana and from 216.67 spores at 20 DAS to 583.33 spores at 80 DAS in Abohar, whereas in Pathogen/AMF, the spore population increased from 258.33 spores at 20 DAS to 670.33 spores at 80 DAS in Ludhiana and from 166.67 spores at 20 DAS to 516.67 spores at 80 DAS in Abohar. No AMF spores were found in the treatment where only the pathogen was present at both locations. Pre-application of AMF with optimal phosphorus levels significantly increased both colonization and spore population in the presence of the pathogen, compared to post-application, as it fostered a stronger symbiotic relationship with the plants. Taffouo et al. (2014) demonstrated that cowpea plants receiving less phosphorus exhibited greater root colonization than those receiving more phosphorus, observed during both vegetative and pod-filling stages. Temegne et al. (2017) observed that mycorrhization

Table 2. Colonization of *Gigaspora margarita* in roots of pea plants in the presence and absence of *Fusarium oxysporum* f. sp. *pisi* at Abohar

Treatment	Colonization percentage after days of sowing			
	20	40	60	80
Mycorrhizal effect				
AMF	25.71 (30.45)	38.27 (38.20)	55.00 (17.85)	71.45 (57.68)
AMF/Pathogen	20.88 (27.18)	32.75 (34.89)	48.00 (43.84)	61.44 (51.59)
Pathogen/AMF	17.04 (24.37)	28.12 (32.01)	40.75 (39.65)	55.30 (48.02)
Pathogen	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD (p=0.05)	0.76	0.73	0.70	0.60
Phosphorus Effect				
P_0	11.72 (20.01)	19.96 (26.52)	30.02 (33.21)	41.38 (40.02)
P_{40}	19.75 (26.37)	29.01 (32.57)	40.79 (39.68)	52.25 (46.27)
P_{80}	16.24 (23.76)	25.38 (30.24)	36.71 (37.31)	47.52 (43.56)
CD (p=0.05)	0.92	0.72	0.65	0.47
Interaction effect between CD (p=0.05)				
P_0 AMF	20.95 (27.22)	31.40 (34.07)	47.25 (43.41)	61.72 (51.76)
P_{40} AMF	31.22 (33.94)	45.09 (42.16)	62.25 (52.08)	79.80 (63.31)
P_{80} AMF	24.95 (29.95)	38.31 (38.22)	55.75 (48.28)	72.84 (58.57)
P_0 AMF/Pathogen	14.32 (22.22)	25.82 (30.52)	39.25 (38.78)	54.29 (47.44)
P_{40} AMF/Pathogen	26.81 (31.16)	38.55 (38.36)	55.50 (48.14)	68.21 (55.66)
P_{80} AMF/Pathogen	21.51 (27.62)	33.87 (35.58)	48.50 (4.12)	61.83 (51.82)
P_0 Pathogen/AMF	11.62 (19.92)	22.62 (28.38)	34.00 (35.65)	49.51 (44.70)
P_{40} Pathogen/AMF	20.99 (27.24)	32.40 (34.68)	45.50 (42.40)	60.98 (51.98)
P_{80} Pathogen/AMF	18.52 (25.46)	29.35 (32.79)	42.50 (40.67)	55.40 (48.08)
P_0 Pathogen	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
P_{40} Pathogen	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
P_{80} Pathogen	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD (p=0.05)	1.20	0.94	0.93	0.98

intensity in *Voandzou* as highest at 30mg of P_2O_5, while higher phosphorus doses did not significantly influence mycorrhization frequency.

Effect of AMF *Gigaspora margarita* on fusarium wilt of pea: At 20 DAS, 100 per cent disease inhibition was observed in the AMF/pathogen treatment across all phosphorus levels in both locations. In the pathogen/AMF treatment, disease inhibition percentages of 90.00, 93.34 and 86.66 were recorded at P_0, P_{40} and P_{80} in Ludhiana (Table 5), respectively. Similarly, in Abohar disease inhibition percentages were 83.33, 93.33 and 80.00 at the same phosphorus levels (Table 6). Plants inoculated solely with the pathogen died at the seedling stage across all phosphorus levels. In treatments with only AMF, 100 per cent of AMF-inoculated plants remained healthy throughout all four observations during the crop season. At 40 DAS, disease inhibition percentages in the AMF/pathogen treatment were 90.00, 96.67 and 86.67 in Ludhiana, and 80, 83.33 and 73.33 in Abohar at P_0, P_{40} andP_{80}, respectively.

At 60 DAS, the AMF/pathogen treatment showed disease inhibition percentages of 83.34, 93.34and 76.67 in Ludhiana, and 76.67, 80.00 and 70.00 in Abohar at P_0, P_{40} and P_{80}, respectively. The pathogen/AMF treatment per cent disease inhibition of 76.67, 83.33 and 70.00 inhibition in Ludhiana, and 66.67, 70.00 and 60.00 in Abohar for the same phosphorus levels. At 80 DAS, the AMF/pathogen treatment showed disease inhibition of 76.67 per cent at P_0, 86.67 at P_{40} and 66.67 per cent at P_{80} in Ludhiana, while in Abohar, the disease inhibition of 70.00 per cent, 73.33 per cent and 66.67 per cent at the corresponding phosphorus levels.

The present findings were corroborated with study conducted by Singh et al. (2019) which indicated that dual inoculation of AMF and pathogen in the presence of phosphorus reduced dry root rot disease intensity in

Table 3. Spore population of *Gigaspora margarita* in rhizosphere of pea plants in the presence and absence of *Fusarium oxysporum* f. sp. *pisi* at Ludhiana

Treatment	Spore population observed after days of sowing			
	20	40	60	80
Mycorrhizal effect				
AMF	333.33	512.78	670.44	778.67
AMF/Pathogen	252.78	441.78	558.02	650.67
Pathogen/AMF	211.11	340.17	422.93	560.67
Pathogen	0.00	0.00	0.00	0.00
CD (p=0.05)	15.26	4.95	6.56	7.67
Phosphorus Effect				
P_0	168.75	236.46	331.00	417.00
P_{40}	235.41	422.75	502.00	574.67
P_{80}	193.75	311.83	405.67	500.33
CD (p=0.05)	13.42	4.39	5.92	7.02
Interaction effect between CD (p=0.05)				
P_0 AMF	291.67	401.00	561.00	673.67
P_{40} AMF	391.67	626.67	786.00	864.33
P_{80} AMF	316.67	510.67	664.00	796.67
P_0 AMF/Pathogen	216.67	317.33	444.67	533.33
P_{40} AMF/Pathogen	291.67	588.00	684.00	763.33
P_{80} AMF/Pathogen	250.00	420.00	546.00	655.33
P_0 Pathogen/AMF	166.67	227.50	318.67	461.33
P_{40} Pathogen/AMF	258.33	476.33	538.67	670.33
P_{80} Pathogen/AMF	208.33	316.67	411.67	550.00
P_0 Pathogen	0.00	0.00	0.00	0.00
P_{40} Pathogen	0.00	0.00	0.00	0.00
P_{80} Pathogen	0.00	0.00	0.00	0.00
CD (p=0.05)	19.98	7.04	9.44	10.85

Table 4. Spore population of *Gigaspora margarita* in rhizosphere of pea plants in the presence and absence of *Fusarium oxysporum* f. sp. *pisi* at Abohar

Treatment	Spore population observed after days of sowing			
	20	40	60	80
Mycorrhizal effect				
AMF	258.33	422.22	536.11	660.56
AMF/Pathogen	186.11	344.45	444.44	544.44
Pathogen/AMF	136.11	291.67	380.56	472.22
Pathogen	0.00	0.00	0.00	0.00
CD (p=0.05)	21.73	15.03	7.85	17.33
Phosphorus Effect				
P_0	118.75	229.17	304.17	395.83
P_{40}	179.17	308.33	379.17	482.92
P_{80}	137.50	256.25	337.50	435.42
CD (p=0.05)	11.02	13.81	5.88	7.49
Interaction effect between mycorrhiza and pathogen				
P_0 AMF	208.33	358.33	466.67	583.33
P_{40} AMF	333.33	516.67	616.67	756.67
P_{80} AMF	233.33	391.67	525.00	641.67
P_0 AMF/Pathogen	158.33	308.33	408.33	491.67
P_{40} AMF/Pathogen	216.67	383.33	483.33	583.33
P_{80} AMF/Pathogen	183.33	341.67	441.67	558.33
P_0 Pathogen/AMF	108.33	250.00	341.67	433.33
P_{40} Pathogen/AMF	166.67	333.33	416.67	516.67
P_{80} Pathogen/AMF	133.33	291.67	383.33	466.67
P_0 Pathogen	0.00	0.00	0.00	0.00
P_{40} Pathogen	0.00	0.00	0.00	0.00
P_{80} Pathogen	0.00	0.00	0.00	0.00
CD (p=0.05)	21.07	19.86	19.86	21.34

Table 5. Effect of *Gigaspora margarita* inoculation on development of pea wilt caused by *Fusarium oxysporum* f. sp. *pisi* at various stages of plant growth and phosphorus level at Ludhiana

Phosphorus level	Treatments	Disease Inhibition % observed after days of sowing			
		20 days	40 days	60 days	80 days
P_0	AMF	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)
	AMF/Pathogen	100.00(89.96)	90.00(74.92)	83.34(66.12)	76.67(61.20)
	Pathogen/AMF	90.00(74.92)	83.33(66.12)	76.67(61.90)	66.67(54.76)
	Pathogen	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
	CD (p=0.05)	7.31	7.81	3.26	5.51
P_{40}	AMF	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)
	AMF/Pathogen	100.00(89.96)	96.67(83.82)	93.34(77.68)	86.67(68.83)
	Pathogen/AMF	93.34(77.68)	90.00(74.92)	83.33(66.12)	73.33(58.98)
	Pathogen	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
	CD (p=0.05)	5.78	7.88	6.31	5.05
P_{80}	AMF	100.00(89.96)	100.00(89.96)	100.00(89.96)	100.00(89.96)
	AMF/Pathogen	100.00(89.96)	86.67(68.83)	76.66(61.20)	66.67(54.76)
	Pathogen/AMF	86.66(68.83)	76.67(61.90)	70.00(56.77)	60.00(50.75)
	Pathogen	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
	CD (p=0.05)	6.26	6.6	3.19	2.94

Table 6. Effect of *Gigaspora margarita* inoculation on development of pea wilt caused by *Fusarium oxysporum* f. sp. *pisi* at various stages of plant growth and phosphorus level at Abohar

Phosphorus level	Treatments	Disease Inhibition % observed after days of sowing			
		20 days	40 days	60 days	80 days
P_0	AMF	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
	AMF/Pathogen	100.00 (89.96)	80.00 (63.41)	76.67 (61.2)	70.00 (56.77)
	Pathogen/AMF	83.33 (66.12)	73.33 (58.98)	66.67 (54.76)	60.00 (50.75)
	Pathogen	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	CD (p=0.05)	3.73	4.52	5.74	4.02
P_{40}	AMF	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
	AMF/Pathogen	100.00 (89.96)	83.33 (66.12)	80.00 (63.52)	73.33 (58.98)
	Pathogen/AMF	93.33 (77.68)	76.67 (61.69)	70.00 (56.77)	66.67 (54.76)
	Pathogen	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	CD (p=0.05)	7.26	3.29	5.94	3.69
P_{80}	AMF	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
	AMF/Pathogen	96.67 (83.82)	73.33 (58.98)	70.00 (56.77)	66.67 (54.76)
	Pathogen/AMF	80.00 (63.41)	63.33 (52.75)	60.00 (50.75)	53.33 (46.90)
	Pathogen	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	CD (p=0.05)	6.44	4.38	3.32	3.59

mungbean plants by 70 per cent in spring and 86.6 per cent in the *Kharif* season if AMF was present before the pathogen. Sarita et al. (2022) observed 100 per cent wilt disease in chili plants when *Fusarium oxysporum* was inoculated alone, but the disease incidence reduced to 60 per cent when co-inoculated with *G. intraradices*.

CONCLUSION

The results highlight that disease development was lower in plants inoculated with both arbuscular mycorrhizal fungus and pathogen compared to only pathogenic treatment at both locations.

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Comprehensive Analysis of Anti-microbial Activity in *Nerium oleander* L. Latex Extracts

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Abstract: This study explored the antibacterial and antifungal potential of *Nerium oleander* latex extracts. In antibacterial tests, *Staphylococcus aureus* and *Pseudomonas aeruginosa* exhibited superior inhibition zones in acetone extracts, while *Bacillus subtilis* and *Proteus vulgaris* showed inhibition in diethyl ether extracts. The diethyl ether extract surpassed the standard drug tetracycline against *Proteus vulgaris*. All extracts demonstrated maximum inhibition at a 15% concentration. In antifungal evaluations against *Aspergillus niger, Fusarium oxysporium*, and *Penicillium sp,* acetone, chloroform, and diethyl ether extracts displayed significant inhibition zones, with concentrations correlating positively with inhibition size. This concentration-dependent relationship indicates *Nerium oleander* latex extracts' potential as a valuable resource for diverse phytopharmacological activities, suggesting promising applications in future disease treatments.

Keywords: *Nerium oleander,* Latex, Antifungal, Antibacterial, Zone of inhibition

Since time immemorial, plants have been the source of medicine throughout the world and still continue to occupy an important place in traditional as well as modern systems of medicine. Even today, rural and tribal communities depend heavily on biodiversity for human and veterinary healthcare, using traditional knowledge is inherited orally from one generation to the other through trial and error methods (Sinha 1996). Plants are also an important source of the world's pharmaceuticals medicine, which are widely used in the treatment of various skin diseases. Ethno-botany is the study of the direct relationship between humans and plants (Adhikari et al., 2021). India, rich in medicinal plant diversity, offers numerous plant-based remedies that are integral to socio-cultural and spiritual practices (Mary, 2017). These plants are also a key source of pharmaceuticals, yielding compounds with antimicrobial, antibacterial, and antifungal properties (Tapsell 2006, Ghosh et al., 2007).

Nerium oleander L.**,** an evergreen shrub or small tree belongs to Apocynaceae family, is a vital medicinal plant in Indian folk medicine. Its flowers, leaves, bark, and latex contains secondary metabolites like flavonoids, alkaloids, steroids, and cardiac glycosides (e.g., oleandrin and neriine) with significant pharmacological applications. Traditionally, it has been used to treat heart conditions, asthma, epilepsy, cancer, malaria, menstrual pain, leprosy, indigestion, ringworm, and venereal diseases, as well as for inducing abortions. The roots possess healing properties for hemorrhoids and ulcers, while its flowers are used in tinctures. *Nerium oleander* exhibits antidiabetic, anti-inflammatory, hepatoprotective, cardiotonic, antioxidant, antibacterial, and antiviral properties (Yogeshwari et al., 2022). This study focuses on the antimicrobial properties of *Nerium oleander* L. against antibiotic-resistant microorganisms, contributing to advancements in natural product-based drug development.

MATERIAL AND METHODS

Collection and preparation of *Nerium oleander* latex extracts: The latex was obtained from *Nerium oleander* plant in the early morning using capillary action and carefully stored in two separate amber glass screw-cap bottles, each holding around 5 ml. The collected latex was subsequently preserved in a refrigerator at -40°C until required. Working solutions, consisting of acetone, chloroform, diethyl ether, and the plant latex, was prepared based on their respective solubility. Following this, a sequence of dilutions was carried out on the stock solutions, resulting in different concentrations (5%, 10%, and 15%).

Preliminary phytochemical analysis: The plant extracts were subjected to phytochemical analysis to detect the presence of alkaloids, flavonoids, tannins, resins, saponins, cardiac glycosides, and steroids (Okeke et al., 2005).

Collection of bacterial cultures: All the bacteria, i.e., *Staphylococcus aureus (*MTCC 7443), *Bacillus subtilis* (MTCC 121), *Proteus vulgaris*, and *Pseudomonas aeruginosa* (NCIM 2200) used for the detection of antibacterial activity were collected from the Department of Microbiology, Yuvaraja's College, Mysuru, and sub cultured on nutrient agar slants. The nutrient agar high medium was prepared as per the requirement according to the number of plates, and the pH was adjusted to 7. The media were sterilized by autoclave at 121°C for 15 min. After sterilization, the nutrient agar medium was poured into sterile Petri plates under aseptic conditions and allowed for solidification.

Preparation of bacterial inoculums: Two ml volume of double-distilled water was placed in a sterile test tube, and a loop full of bacterial culture was suspended in it. Subsequently, one ml of this sample was inoculated into the agar medium and left for culturing.

Antibacterial activity: The bacterial subculture was evenly spread across the medium using an L-shaped glass rod. Various concentrations of different extracts (acetone, chloroform, and diethyl ether) were applied onto sterile discs with a diameter of 0.1 mm and positioned on the plates. Additionally, a preparation of latex extract, along with the standard drug tetracycline (50 mg/ml), was impregnated onto separate discs to serve as the standard for assessing antibacterial activity. Subsequently, the plates were incubated overnight at 37°C (Harborne 1973).

Antifungal assay: Fungal cultures, including *Aspergillus niger, Penicillium* sp, and *Fusarium oxysporum,* were acquired from the Department of Microbiology at Yuvaraja's College, Mysuru. These cultures were sub cultured in potato dextrose broth solution for subsequent applications. A potato dextrose agar high medium was formulated as per specifications and autoclaved at 121°C for 15 minutes. Following sterilization, the potato dextrose agar medium was carefully poured into sterile Petri plates under aseptic conditions and allowed to solidify.

Preparation of fungal inoculum: One ml aliquot of the fungal inoculum from the sub cultured broth solution is transferred to a sterile test tube, which is then adjusted to a total volume of two ml with sterile distilled water. The mixture is thoroughly shaken. Subsequently, one ml of this inoculum is applied to the plates for further inoculation.

Antifungal activity: The fungal subculture was evenly distributed across the medium using an L-shaped glass rod. The stock solutions, resulting in different concentrations (10%, 20% and 30%) were employed for the antifungal activity. Sterile discs with a diameter of 0.1 mm were impregnated with various concentrations of different extracts (acetone, chloroform, and diethyl ether) and positioned on the plates. Additionally, a preparation of latex extract, along with the standard drug Fluconazole (50 mg/ml), was impregnated onto separate discs to serve as the control for assessing antifungal activity. The treated plates were then incubated at 30°C for 24-48 hours. Each experiment was conducted in triplicate for accuracy and reliability.

RESULTS AND DISCUSSION

In the present study to assess antibacterial activityof *N. oleander* latex extracts were tested against *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris,* and *Pseudomonas aeruginosa* at 5%, 10%, and 15 % of acetone, chloroform, and diethyl ether solvent extracts. All extracts exhibited maximum inhibition zones at a concentration of 15% (Table 1, Fig. 1). It was reported that diethyl ether extract of latex of *E. heterophylla* exhibited significant antibacterial activity against *S. aureus* and *P. aeruginosa* (Pruthvi et al., 2020). In present study acetone latex extract demonstrated the highest potential zone against *Staphylococcus aureus* compared to chloroform and diethyl ether extracts. Similarly, diethyl ether latex extract showed the highest potential against *Bacillus subtilis* compared to chloroform and acetone

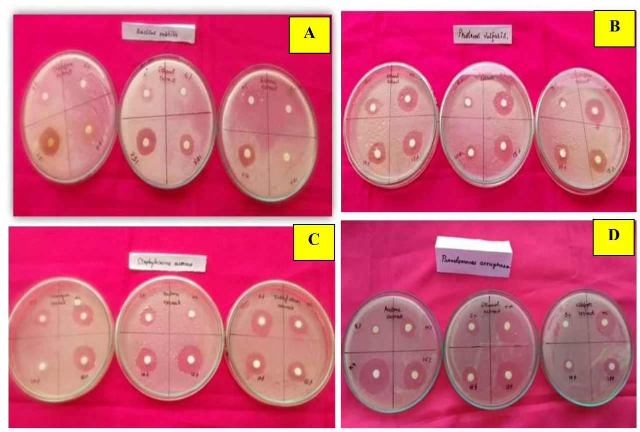

Fig. 1. Antibacterial activity of *N. oleander* latex extracts against (A) *Bacillus subtilis,* (B) *Proteus vulgaris,* (C) *Staphylococcus aureus and* (D) *Pseudomonas aeruginosa*

Table 1. Antibacterial activity of *Nerium oleander* latex extracts against four Bacterial strains

Bacterial strains	Acetone			Chloroform			Diethyl ether latex			+ve control (Tetracycline) 5%		
	5%	10%	15%	5%	10%	15%	5%	10%	15%			
Bacillus subtilis	0.0	0.0	6.0	0.0	0.0	8.0	0.0	6.0	9.0	6.0	10.0	4.0
Proteus vulgaris	2.0	4.0	10.0	3.0	6.0	8.0	6.0	7.0	12.0	7.0	8.0	8.0
Staphylococcus aureus	7.0	8.0	13.0	0.0	4.0	9.0	5.0	8.0	11.0	12.0	11.0	12.0
Pseudomonas aeruginosa	4.0	7.0	10.0	0.0	3.0	6.0	3.0	6.0	10.0	14.0	9.0	12.0

Table 2. Antifungal activity of *Nerium oleander* latex extracts against three fungal strains

Bacterial strains	Acetone latex extract			Chloroform latex extract			Diethyl ether latex extract			+ve control (Fluonozole) 5%		
	5%	10%	15%	5%	10%	15%	5%	10%	15%			
Aspergillus niger	2.0	4.0	7.0	3.0	5.0	7.0	3.0	4.0	8.0	10.0	12.0	12.0
Fusarium oxysporum	4.0	6.0	8.0	3.0	5.0	6.0	2.0	4.0	7.0	10.0	9.0	6.0
Penicillium sp.,	3.0	4.0	6.0	0.0	3.0	5.0	0.0	4.0	6.0	9.0	6.0	7.0

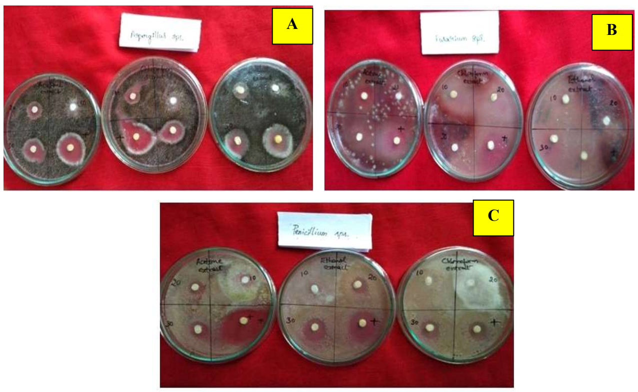

Fig. 2. Antifungal activity of *N. oleander* latex extracts against (A) *Aspergillus niger,* (B) *Fusarium oxysporum* and (C) *Penicillium* sp.

extracts. The acetone, chloroform, and diethyl ether latex extracts displayed consistent inhibition zones, diethyl ether latex extract exhibited the highest inhibition zone against *Proteus vulgaris*. The diethyl ether extract displayed a remarkable inhibition zone compared to the standard drug tetracycline used as a control. Similar potential zones were observed in diethyl ether and acetone latex extracts against *Pseudomonas aeruginosa* compared to chloroform latex extract. At concentrations of 900 mg/ml and 500 mg/ml, *Nerium oleander* exhibited inhibition zones of 22 mm and 13 mm, respectively, against *Staphylococcus aureus* (Wong et al., 2013). Notably, a remarkable zone of inhibition was observed in the diethyl ether extract when compared to the standard drug tetracycline, used as a control, particularly in tests against *Proteus vulgaris.*

The antifungal efficacy of *N. oleander* latex was assessed using the disc diffusion method against various fungal strains. The plant extracts exhibited substantial zones of inhibition against *Aspergillus niger, Fusarium oxysporium*, and *Penicillium sp*. (Table 2, Fig. 2). The acetone, chloroform, and diethyl ether *N. oleander* latex extracts demonstrated significant zones of inhibition against *Aspergillus niger*. The zone of inhibition increased proportionally with the concentration of the latex extract, indicating a concentration-dependent effect. Acetone latex extract exhibited a particularly high potential zone of inhibition. When tested against *Fusarium oxysporium*, an increase in the zone of inhibition was observed with a higher concentration of *N.oleander* latex extract in all three solvent, acetone extract displayed a higher potential zone of inhibition compared to chloroform and diethyl ether extracts. The broad spectrum of antibacterial and antifungal activities demonstrated by *N. oleander* latex suggests the potential discovery of new chemical classes of antibiotic substances. These findings could contribute to the development of alternatives or second-line treatments for infectious diseases and their control.

CONCLUSION

The antimicrobial activity assessment demonstrates the ability of *Nerium oleander* latex to inhibit the growth of bacteria and fungi. These findings collectively provide valuable insights into the potential therapeutic applications of *N. oleander* latex, suggesting its possible use in the development of antimicrobial agents for treating various diseases. Further research and clinical studies would be essential to explore and validate its efficacy and safety in practical therapeutic applications.

AUTHORS' CONTRIBUTIONS

Dr. Sahaya Mary and Kavitha RS authored the research and contributed to the preparation of the manuscript. Dr. Mahesh provided guidance on the manuscript structure and contributed to the development of the methodology

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GC-MS and ICP-OES Analysis of Secondary Metabolites From Traditional Rice Varieties of Andhra Pradesh, India

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***Abstract*:** The popular varieties such as Navara, Parimala Sanna, Bahurupi, Sugandhi, and Indrani are commonly cultivated in Andhra Pradesh. Traditionally germinated red, black and brownrice has been used in Asian traditional medicine for centuries. Hence the present study is aimed to find out the metabolites from selected varieties by using standard methods. Qualitative analysis revealed the presence of a greater number of metabolites from aqueous extracts. The quantitative analysis highlighted the significant levels of flavonoids, proteins, carbohydrates, ascorbic acidand fatty acids from Parimala Sanna and Navara varieties. The GCMS analysis of Parimala Sanna aqueous extract revealed that the presence of 1,2,2-trichloroethane, 1,1-difluoro ethane, 1,2,5-oxadiazole, 4 methyl 4-pentane 2-one, formic acid, ethyl ester, 3-hydroxy-2 butanone with higher percentage. ICP OES analysis showed that the Sugandhi variety isricher in Iron, Sodium, Potassium, Sulphur and Copper than that of other varieties. This study will be helpful for isolation and identification of active principles from these rice varieties.

***Keywords*:** *Oryza sativa*, Phytochemicals, GC-MS, ICP-OES, Secondary metabolites, Medicinal properties

Oryza sativa (Poaceae) is the most widely consumed staple food for a large part of world human populations in Asia. Traditional colored rice varieties are particularly known for their high dietary fiber, starch, flavonoids, phenols, and other beneficial compounds, that are believed to contribute to reducing the incidence of non-communicable diseases such as cardiovascular disease, diabetes, cancer, and stroke (Bhattacharyya and Roy 2018). De- husked rice has higher protein, fiber, minerals, and vitamins compared to non-medicinal varieties. It is well-suited for use in traditional Ayurvedic therapy and is known for facilitating the transfer of bioactive compounds from medicinal herbs and maintaining heat during topical body massage (Deepa et al., 2007).

Paddy is cultivated in an area of about 155 million hectares with a production of about 596 million tonnes that provides 22 per cent of the world's supply of calories and 17% of the proteins. India ranks second in production with 131 million tonnes of paddy next to China with average yield per hectare whereas, India extends from 8 to 35°N latitude and from sea level to as high as 3000 meters. Rice is a short-day plant that needs a hot, humid climate and an assured supply of water. The average temperature required throughout the life period of the crop ranges from 21 to 37°c. The crop requires a high temperature for blooming, in the range of 26.5 to 29.5°C at the time of tillering than ripening. Soils having good water retention capacity with a good amount of clay and organic matter are ideal for rice cultivation. Clay or clay loams are most suited for rice cultivation, such soils are capable of holding water for long and sustain crops. Rice being a semi-aquatic crop grows best under submerged conditions. Rice plants are able to tolerate a wide range of soil reactions, but it does have a preference for acidic soils. It grows well in soils having a pH range between 5.5 and 6.5. It can be grown on alkali soils also, after treating them with gypsum (Ranking analytics 2024). Cultivars with growth duration of 150 to 210 days are usually photoperiod sensitive and planted in the deep water areas. Temperate day length are two environmental factors affecting the development of the rice plant that can be divided into three phases. Rice grains have been acquiring more attention from nutritionists, consumers, and health consultants in the past few years due to their greater importance in their biological activity, nutritional value, and substantial impact on human health. Due to its higher digestibility, nutritional quality, potential health, and biological activity, rice is categorized as the queen of cereal (Verma et al., 2017). Moreover, a large percentage of the Asian population heavily relies on rice as their primary energy source of food, mainly carbohydrates and a small amount of protein, recent studies have highlighted the nutritional value of rice protein (Juliano1985 and Chaudhary and Tran 2001). The traditional varieties are a viable source of various agricultural properties as well as sources of many bioactive non-vital nutrients such as vitamin D, calcium, thiamine, riboflavin, glutamic acids, and high in fiber (Bhat and Riar 2015; Verma et al., 2020). Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is an analytical technique used to determine nutritional elements in a sample (Bulska and Wagner 2016).

Medicinal rice like Njavara contains high levels of minerals and carbohydrates and is used in treating various diseases such as arthritis, muscle wasting, and neurological disorders. Phytochemicals are bioactive compounds with antioxidant properties that are essential for safeguarding the body from diseases by delaying or inhibiting cellular damage. Medicinal plants also exhibit significant antioxidant potential due to their diverse phytoconstituents. In contrast, concerns have been raised about the potential negative health effects of synthetic antioxidants, leading to strict restrictions on their use. As a result, there is a growing trend to replace synthetic antioxidants with naturally occurring ones (Ankanna and Savithramma 2011). Rice bran have several pharmacological activities including immunomodulatory, antidiabetic, anti-inflammatory, antioxidant, anticancer, cardiovascular protective, anti-hyperlipidemic, hepatoprotective, nephroprotective, and antimicrobial, making them potential components to act as a nutraceutical with therapeutic value (Sen et al., 2020).Gas chromatography-mass spectroscopy (GC-MS) is a combined analytical technique used to determine and identify compounds present in a plant sample (Uma and Balasubramaniam 2012). It plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active compounds (Hethelyi et al., 1987). Furthermore, phytochemical compounds tend to accumulate in the pericarp and bran of the rice kernel. Studies have shown that rice varieties with color pigments, such as black rice, are rich in anthocyanin and other polyphenolic compounds (Muntana and Prasong 2014). The Indian Materia Medica, an Ayurvedic treatise, mentions several medicinal rice varieties in India, highlighting the need to document and conduct research on these Rice varieties (Das and Oudhia 2003). The scientific data on the nutritional and medicinal properties of popular rice varieties in India, such as Navara, Parimala Sanna, Bahurupi, Sugandhi, and Indrani, are scanty. Therefore, the present study aimed to screen primary and secondary metabolites in these rice varieties were carried out by using standard methods both qualitative and quantitatively.

MATERIAL AND METHODS

Procurement of raw materials: The study included five representative rice varieties (Fig. 1), procured from diverse regions of India. Navara rice was cultivated in Kerala, while the other varieties Parimala Sanna, Bahurupi, Sugandhi and Indrani were obtained from farmers in Andhra Pradesh.

Sample Preparation: The samples were carefully cleaned to eliminate small sand particles and impurities before being ground into a fine powder. This powder was then sifted through a 0.5 mm metallic mesh to obtain a crude fine powder suitable for phytochemical screening using standard chemical tests and nutritional properties were estimated by ICP OES method and GCMS.

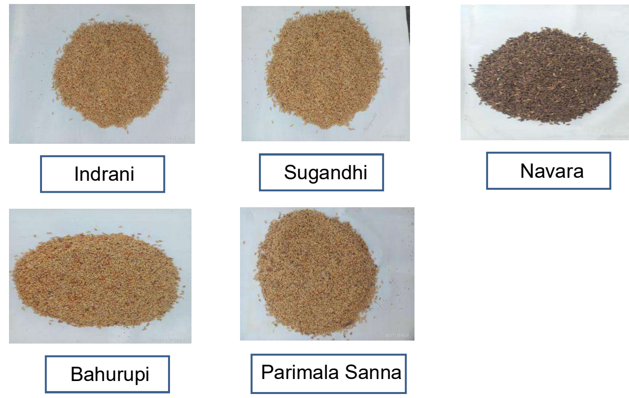

Fig. 1. Selected rice varieties

Extraction: Extractions were carried out using polar solvents such as water and methanol, as well as nonpolar solvents including benzene and hexane. For aqueous extraction, 5g of each rice powder was dissolved in 100 ml of distilled water and boiled in a water bath for 30 minutes. The mixture was filtered, and the filtrate was stored for further processing. The remaining solvents were used for extraction by macerating 5g of each powder with 100 ml of each solvent for 24 hrs, followed by filtration and storage (Peeriga and Banoth 2016 and De Silva et al., 2017).

Phytochemical Screening

Metabolite name	Reference
Carbohydrates	McCready et al., 1950
Sugars	Duboise et al., 1956
Lipids	Jayaraman 1981
Proteins	Lowry et al., 1951
Tannins	Van-Burden and Robinson 1981
Saponin	Obadoni and Ochuko 2002
Lignin	Gibbs 1974
Tannins	Trease and Evans 1985
Anthocyanins	Paris and Moyse 1969
Leucoanthocyanins	Paris and Moyse 1969
Flavonoids	Peach and Tracey 1956
Steroids	Gibbs 1974
Tannins	Trease and Evans 1985
Glycosides	Harborne 1973
Alkaloids	Gibbs 1974
Phenols	Gibbs 1974
Emodines	Harborne 1973
Ascorbic acid	Fujita et al., 1935

Screening for metabolites from selected rice varieties: This was done as per standard procedure (Table 1).

Statistical analysis: Statistical analysis was performed using KyPlot version 2.0 beta 15 (32 bit).

RESULTS AND DISCUSSION

The phytochemical study of different rice varieties revealed that the aqueous extract of all rice grain varieties contained a greater number of compounds, primary metabolic compounds like carbohydrates, proteins, reducing sugars, ascorbic acid and fatty acids; and secondary metabolites like alkaloids, flavonoids, glycosides, terpenoids, steroids, tannins, and coumarins (Table 1). The hexane extract showing fewer compounds compared to methanol and benzene. The methanolic extracts showed moderate primary and secondary compounds in Parimala Sanna and Sugandhi, whereas, Navara showed more compounds but Indrani and Bahurupi having fewer compounds. Benzene extracts of all varieties possess proteins and Bahurupi showed carbohydrates, fatty acids and reducing sugars but Indrani had fatty acids only. In addition to primary metabolites alkaloids, tannins and glycosides presence in Parimala Sanna whereas Sugandhi and Indrani indicated almost equal compounds but Navara revealed lower compounds. Hexane extracts ofall varieties contained tannins and alkaloids but in addition to these, Parimala Sannahad fatty acids.

The aqueous extracts showed more solubility of secondary metabolites than other solvents such as methanol, benzene and hexane. Among all tested varieties Navara and Parimala Sanna have a greater number of secondary metabolites followed by Sugandhi, Bahurupi and Indrani. Primary metabolites like proteins, reducing sugars and carbohydrates were present in a greater number of solvents. Whole grain rice has a rich nutritional profile and medicinal properties (Carlos et al., 2007) and plays a crucial role in human health and provides numerous health benefits (Valarmathi et al., 2014).

Parimala Sanna hashigher content of flavonoids, carbohydrates and ascorbic acid than the other varieties (Table 2) andSugandhi only high protein content. The nutrients and phytochemicals present in rice bran are comparable to those found in other whole grain cereals like corn, oat and wheat, providing disease protection and immune system support (Baris and Yilmaz 2011). Certain traditional rice varieties such as Kattuyanam, Mapillai Samba, Navara, Karunguruvai, Kavuni, Kichadi Samba, Illupaipoo Samba, Kalana Mak, Karudan Samba and Seeraga Samba can help treat various human ailments and beneficial physiological changes in the body (Kowsalya et al., 2022). Many rice varieties are used in medical purposes such as atropine as anticholinergic and morphine as analgesic. A small amount of alkaloids is present in black rice while it is completely absent in Gobindobhog rice (Bhattacharyya and Roy 2018). The bran and germ regions of rice are the nutrient-dense whereas outer layers and sprouting parts of the grain are rich in antioxidants, phytochemicals and other beneficial compounds (Ghasemzadeh 2018). The bioactive compounds concentrated in the bran regions of rice have numerous health benefits, including reducing inflammation

Table 1. Preliminary screening of phyto-metabolites from selected rice varieties

	Bahurupi				Parimala Sanna				Sugandhi				Indrani				Navara			
	A	M	B	H	A	M	B	H	A	M	B	H	A	M	B	H	A	M	B	H
Carbohydrates	+	-	-	-	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-
Proteins	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
Reducing sugars	+	+	+	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
Fats	+	-	+	-	+	-	-	+	+	-	-	-	+	-	+	-	+	-	-	-
Alkaloids	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	-	-	+	+	-	-	-	+	+	-	-	-	+	-	+	+	-	-
Tannins	-	-	-	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-	+
Glycosides	+	-	+	-	+	+	+	-	-	+	+	-	+	-	+	-	+	+	+	-
Saponins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coumarins	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Terpenoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascorbic acid	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-

A=Aqueous, M=Methanol, B= Benzene, H= Hexane; '+' indicates Presence, '-'Indicates Absence

Table 2. Quantitative estimation of metabolites from selected rice varieties

Compounds	Flavonoids	Proteins	Carbohydrates	Lipids	Ascorbic acid
Bahurupi	0.12±0.01	0.8±0.14	18.8±0.63	0.12±0.1	6.5±0.20
ParimalaSanna	0.13±0.01	1.33±0.04	20.6±0.18	0.01±0.01	6.5±0.06
Sugandhi	0.02±0.01	1.75±0.30	11.11±0.52	0.05±0.02	0.43±0.04
Indrani	0.10±0.01	1.2±0.02	9.7±0.42	0.06±0.01	13±0.20
Navara	0.02±0.01	0.84±0.01	20.2±0.17	0.05±0.01	6.5±0.12

± indicates the SE of three samples

Table 3. GCMS analysis compounds in Parimala Sanna rice

Name of the compound	RT	Area %	MF	MW (g/mol)	Biological activity
1,2,2 trichloroethane	4.29	1.16	$C_2HC_{13}F_2$	168	Anesthetic, Analgesic, Sedative
2,2- dimethoxy propane	4.29	1.16	$C_2H_4F_2$	64.05	Analgesic, Sedative
1,2,5 oxadiazole	3.77	0.59	$C_2H_2N_2O$	70	Anti-inflammatory, Anti-bacterial, Anti-viral, Anti-cancer
4 methyl 1,4 pentane 2 one	3.63	0.69	$C_6H_{10}O$	98	Anti-inflammatory, Anti-bacterial, Anti-cancer, Antioxidant
Formic acid	3.25	0.77	$C_3H_4O_2$	72	Anti-inflammatory, Anti-bacterial, Antiseptic, Antifungal
Ethyl ester (Ethyl acetate)	3.25	0.77	H_5COOR	88.12	Analgesic, Antioxidant
3 hydroxy 2 butanone	3.09	0.75	$C_4H_8O_2$	88	Anti-inflammatory, Anti-bacterial, Antifungal

RT: Retention time, MF: Molecular formula,MW: Molecular weight

and improving cardiovascular health (Penny et al., 2002). Traditional rice contains a range of bioactive compounds, which are present in small quantities in various fractions of the grain. These bioactive compounds exhibit diverse biological activities (Rondanelli et al., 2019).

Sugandhi showed a greater number of nutritional properties like iron, sodium, potassium, sulphur and copper than the other varieties, but Navara recorded higher volume of phosphorus and in than the other varieties. Rice is a good source of essential minerals that contains significant amounts of magnesium, phosphorus and calcium as well as trace elements like iron, zinc, copper and manganese (Verma et al., 2017 and 2020). Traditional rice is an essentialnutritiousfood and is an excellent source of vitamin-D, calcium, thiamine, riboflavin, glutamic acid and fibre (Bhat and Riar, 2015). The rice bran contains a range of nutrients, including vitamin B9, essential amino acids and micronutrients (Goffman and Bergman 2004). The GCMS analysis revealed that a total 60 compounds were identified in aqueous extract of Parimala Sanna (Table 3). Among these 1,2,2-trichloroethane, 1,1-difluoro ethane, 1,2,5-oxadiazole, 4 methyl 4-pentane 2-one, formic acid, ethyl ester, 3-hydroxy-2 butanone showed higher percentage. Chandra Sekar and Bhagavathy (2023) reported the same results in Rice varieties like Karuppu kavuni, Mappillai samba and Seeraga samba of south India. ICP-OES analysis revealed that, among all rice varieties, Sugandhi are rich iron, sodium, potassium, sulphur and copper (Table 4). Valarmathi et al. (2014) reported the same results in Kavuni in traditional rice variety of Tamil Nadu. These findings are in line with previous reports that emphasize differences in the activities of extracts obtained from the same plant using different solvents.

Table 4. ICP-OES identification of nutrient components in Parimala Sanna

Nutrients	mg/ Kg dry wt
Sodium (Na)	1.34
Potassium (K)	1.44
Phosphorus (P)	0.26
Sulfur (S)	0.04
Calcium (Ca)	0.19
Magnesium (Mg)	0.03
Copper (Cu)	24.5
Manganese (Mn)	54.3
Iron (Fe)	172.5
Zinc (Zn)	33.9

CONCLUSION

Rice varieties of Parimala Sanna and Sugandhi have many bioactive compounds that may help to regain immune power and maintain various metabolic reactions inside the body to dominate over a wide range of stress generated ailments due to their free radicals. Results indicate that the presence of various types of phytochemicals in aqueous extracts. Water is the best solvent for nutritional and pharmaceutical preparations from rice varieties.

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Integrated Biochemical Analysis of Red Rice (*Oryza sativa* L.): Unveiling Nutritional and Functional Attributes

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Abstract: The present study evaluates the physicochemical and nutritional characteristics of red rice varieties collected from different districts of Himachal Pradesh namely Shimla, Kullu, Chamba and Kangra, each representing distinct geographical locations. There were significant differences in the physicochemical and nutritional characteristics of varieties. Grain size classifications showed that Chhohartu, Matali, Jattoo, Karad, Jhinjhan and Kaluna were short, whereas Gocha, HPR-2720 and HPR-2795 were medium. Grain shapes were medium in Chhohartu, Karad, Jhinjhan, HPR-2720 and HPR-2795, while Matali, Jattoo, Kaluna, and Gocha were bold. Amylose content ranged from 18.27% (Karad) to 25.17% (Chhohartu). The proximate composition indicated that Jattoo (13.67%) had the highest moisture content, whereas Kaluna (3.56%) showed the highest ash content. Protein content was high across all the varieties ranging from 8.66% (Jhinjhan) to 13.89% (Gocha). Jhinjhan (402.2 mg/kg) showed the highest iron content, while Karad (83.6 mg/kg) had the lowest. The highest zinc content was observed in Kaluna (46.55 mg/kg) while Matali (32.35 mg/kg) had the lowest. The significant negative correlation was observed between carbohydrate and protein (r= -0.810**), energy and moisture (r = -0.877**), and a moderate negative correlation between energy and ash (r = -0.711*), indicating that higher moisture and ash content in red rice varieties correspond to lower energy content. The current research demonstrated that the variations observed in these characteristics are influenced by environmental factors across the different geographical regions.

Keywords: Red rice, Himachal Pradesh, Physicochemical characteristics, Nutritional parameters

Red rice (*Oryza sativa* L.), known for its unique red bran layer, is gaining significant attention for its remarkable nutritional and functional properties. A distinctive feature of this type of rice is that even after extensive milling, a slight red remains in the outermost layer. The bran colour, varying from light to dark red, is caused by anthocyanin and proanthocyanidin pigments in different bran layers. It is an Asian native that has long been grown in the Philippines, Japan, China, Sri Lanka, Korea, India, and other rice-growing countries. The northwest Himalayas regions, such as Uttarakhand, Jammu and Kashmir and Himachal Pradesh, exhibit significant diversity in indigenous rice cultivars. Several well-known red rice cultivars in India are Matta rice from Kerala, Patni from Maharashtra, Jattoo and Matali from Kullu Valley in Himachal Pradesh (Devraj et al., 2020). These red rice varieties are part of both indica and japonica subspecies, exhibiting a range of characteristics such as glutinous or non-glutinous, aromatic or non-aromatic, and early or late maturing. They also vary in grain length, from short to long, although the majority are coarse-grained. Himalayan red, a long-grained red rice variety has gained recognition in the export market (Ahuja et al., 2007).

The nutritional profile of red rice is notably remarkable, as it contains a high concentration of essential nutrients that support overall health and welfare. Shao and Bao (2015) documented that whole grain contains carbohydrates, protein, lipids, dietary fibres, minerals, vitamins (E and B complex) and phytochemicals like phenolic compounds. The primary components of whole grains are carbohydrates, mainly starch. Amylose is the main factor influencing the various physiochemical characteristics of starch (Wani et al., 2012). Moreover, whole grains are higher in dietary fibre, which can be widely divided into soluble and insoluble fibre (Slavin et al., 2003). Protein is a major constituent of red rice after starch, with four distinct fractions: globulin, albumin, glutelin (the predominant protein) and a minor protein prolamin (Amagliani et al., 2017). The third naturally occurring component in red rice is lipids, or fats, mainly located in the outer bran layer and exit in both starch and non-starch-associated forms. Saleh et al., (2019) observed that lipid content significantly influences the quality of rice throughout processing and storage. Micronutrients, including minerals and vitamins, are essential for good health while being required in small quantities. Red rice has a two to three times higher zinc and iron content than white rice. contains magnesium which lowers the risk of heart attacks and plays a critical function in blood pressure regulation. Manganese and calcium are present in abundance which helps in strengthening metabolism, and bones. Selenium is also a part of the nutritional package of red rice and helps the body fight against infection. Red rice's low glycemic index makes it suitable for several conditions, including diabetes. Red rice may also be helpful for the prevention or cure of diseases caused by vitamin A and B deficiencies (Kristamtini et al., 2012).

Red rice pericarp contains a significant amount of phenolic substances (in soluble form) and antioxidants, in addition to its high functional food value (de Mira et al., 2009). Phenolic acids, flavonoids and tannins are considered the primary dietary phenolic compounds (King and Young 1999). Among red rice varieties, the most abundant bound phenolic acid is ferulic acid followed by *p*-coumaric and vanillic acid (Sompong et al., 2011). Cyanidin 3-O-glucoside is the primary anthocyanin found in red rice (Tomoyuki et al., 2002). The flavonoids identified in red rice include proanthocyanidins, which belong to the class of flavanols (Min et al., 2011). Proanthocyanidins, also known as condensed tannins, are the most abundant polyphenols in the plant kingdom and are made up of oligomers of flavan-3-ol and flavan-3,4-diol (Smerglio et al., 2017). The red rice of Himachal Pradesh is poorly understood, and only a few studies have been done on its quality characteristics. The study aims to determine the nutritional and functional attributes of red rice grown in different regions of Himachal Pradesh.

MATERIAL AND METHODS

Sample collection: Seven landraces (Chhohartu from Shimla district, Matali and Jattoo from Kullu district, Karad and Jhinjhan from Chamba district and Gocha and Kaluna from Kangra district) and two released varieties (HPR-2720 and HPR-2795 from Kangra district) of red rice were collected from different regions of Himachal Pradesh during the years 2022 and 2023.

Details regarding the naming of the red rice varieties were obtained from the Wheat and Rice research station, Malan CSKHPKV Palampur (H.P.). The samples were cleaned manually to remove debris and attached soil, followed by shade-drying. After drying, the grains were dehusked using a de-husker (available at the Wheat and Rice research station, Malan), finely ground into a powder with a laboratory grinder and passed through a 60-mesh sieve. The powdered samples were kept in airtight containers to maintain their stability until further analysis was performed. All the experiments were done in triplicate.

Physicochemical characteristics: The size and shape classification were determined by the scale of (Cruz and Kush 2000). The shape of the kernel was assessed by calculating the length-to-width (L/W) ratio. The alkali spreading value (ASV) was assessed using the procedure by Little et al. (1958) with some alternations. The starchy endosperm of the rice kernels was visually rated using the 7-point numerical spreading scale. The amylose content (AC) was measured using the procedure by Juliano et al., (1971) with slight modifications. The amylose content was estimated using the standard curve of potato amylose.

Proximate composition and food energy: The moisture content of the sample was determined using the hot air oven method (AOAC 2000). Ash content was determined by the muffle-furnace method as per the procedure referred to by AOAC (2000). The samples in the crucibles were heated at 550 ℃ for 5-6 hrs in a muffle furnace. The fat content was determined using Soxhlet extraction following the method (AOAC 2005). The extraction was conducted 4-6 hours at 60-68 ℃ temperature. Crude fibre content of the sample was measured by the AOAC (2000) procedure. The crude protein content was analysed using the Kjeldahl method as per the procedure detailed in AOAC (1990), which encompassed protein digestion and distillation.

The total carbohydrate content in the rice sample was calculated (James 1995): =100- (% of Moisture + % of ash + % of fat + % of crude fibre + % of crude protein). Food energy was calculated by the formula of (Verma and Srivastav 2017): Food energy (kJ/100g) = (%CP*4) + (%F*9) + (%CHO*4), Where CP = crude protein, F = fat and CHO = carbohydrate.

Mineral Analysis: The minerals present in the samples were estimated using atomic absorption spectroscopy (AAS). The

Table 1. Collection sites and GPS coordinates of red rice varieties

Varieties	Collection sites	Latitude	Longitude
Chhohartu	Peja, Shimla district	31° 22'72"N	77° 86'25"E
Matali	Prini, Kullu district	32° 21'50"N	77° 19'58"E
Jattoo	Prini, Kullu district	32° 22'34"N	77° 19'70"E
Karad	Kari Chaonri, Chamba district	32° 48'02"N	76° 01'46"E
Jhinjhan	Nagori, Chamba district	32° 56'68"N	76° 11'03"E
Kaluna	Dungla, Kangra district	32° 21'60"N	76° 30'68"E
Gocha	Supara, Kangra district	32° 31'38"N	76° 18'37"E
HPR-2795	Tarindi, Kangra district	32° 10'31"N	76° 35'87"E
HPR-2720	Tarindi, Kangra district	32° 10'31"N	76° 35'87"E

Table 2. Analysis of proximate composition of red rice

Parameters	Methods	References
Moisture	Hot air oven	AOAC 2000
Ash	Muffle furnace	AOAC 2000
Fat	Soxhlet extraction	AOAC 2005
Crude fibre	Acid and alkali digestion	AOAC 2000
Crude protein	Micro Kjeldhal	AOAC 1990
Total carbohydrate	By difference: 100- (Moisture + ash + fat + crude fibre + crude protein)	James 1995

diacid mixture method was used to prepare the samples (Renuka et al., 2016).

Statistical analysis: The data were statistically analysed using Duncan's multiple range tests at the 0.05 significance level and Pearson's correlation coefficients at 0.05 and 0.01 significance levels with SPSS software [Version 29.0.2.0 (20)].

RESULTS AND DISCUSSION

Physicochemical characteristics: Grain size is characterized by its maximum dimension, while grain shape is based on the length-to-breadth ratio. The average grain length ranged from 4.86 (HPR-2795) to 6.30 mm (Kaluna). The average grain width varied between 2.09 (HPR-2720) to 2.81 mm (Matali). The length-to-width (L/W) ratio of red rice varieties varied from 1.88 (Kaluna) to 2.89 (HPR-2720). Chhohartu, Matali, Jatto, Karad, Jhinjhan and Kaluna were short in size, while Gocha, HPR-2720 and HPR-2795 were medium in size. L/W ratio Chhohartu, Karad, Jhinjhan, HPR-2720 and HPR-2795 were medium, while Matali, Jattoo, Kaluna and Gocha were bold in shape. Abeysekera et al., (2017) documented that the grain length of varieties ranged from 4.12 to 5.98 mm and length to width ratio ranged from 1.81 to 3.03.

The alkali spreading value (ASV) of the various red rice varieties ranged from 1.66 (HPR-2720 and HPR-2795) to 5.16 (Karad). The majority of red rice varieties exhibited an intermediate gelatinization temperature (GT). The high GT with low ASV was observed in Kaluna, HPR-2720 and HPR-2795. The intermediate GT and ASV were in Chhohartu, Matali, Jattoo, Karad, Jhinjhan and Gocha. Lahkar et al. (2020) observed a similar range of ASV in aromatic Joha rice, ranging from 1 to 5 most of which exhibited intermediate scores. Amylose content (AC) varied significantly ranged from 18.27 (Karad) to 25.17% (Chhohartu). A study by Thongbam et al. (2010), reported amylose content in rice cultivars from Manipur ranging between 14.33 to 29.47%, most of the cultivars classified as having intermediate amylose content (20-25%).

Proximate composition and food energy: Statistically significant differences were observed in the proximate composition among red rice varieties. The moisture content ranged from 11.56 (Chhohartu) to 13.67% (Jattoo). Saikia et al. (2012) showed similar outcomes in pigmented rice varieties from Manipur ranging from 11.6 (Chak-haoamubi) to 13.7% (Bakuljoha). Ash content varied from 2.27 (Jhinjhan) to 3.56% (Kaluna). Lahkar et al. (2020) reported ash content in traditional aromatic (Joha) rice ranged from 1.0 (Tulsi Joha and Rampal Joha) to 7.47 % (Boga Kunkuni Joha). The fat content ranged from 1.02 (Matali) to 3.46% (Jattoo). Sompong et al., (2011), reported fat content in rice varieties (nine red and three black) varied from 1.15 (Sri Lanka Red rice 1) to 3.72% (Niaw Dam Pleuak Khao). Crude

Fig. 1. Red rice varieties (a) Chhohartu (b) Matali (c) Jattoo (d) Karad (e) Jhinjhan (f) Gocha (g) Kaluna (h) HPR-2795 (i) HPR-2720

Table 3. Physical characteristics of red rice (Mean±SE, n=30)

Varieties	Grain length (mm)	Grain breadth (mm)	L/B ratio	Grain size	Grain shape
Chhohartu	5.49±0.05	2.61±0.03	2.11±0.02	Short	Medium
Matali	5.41±0.04	2.81±0.02	1.93±0.02	Short	Bold
Jattoo	5.17±0.04	2.52±0.02	2.05±0.02	Short	Bold
Karad	5.34±0.06	2.41±0.04	2.23±0.05	Short	Medium
Jhinjhan	5.23±0.03	2.12±0.02	2.46±0.04	Short	Medium
Kaluna	4.86±0.03	2.57±0.01	1.88±0.01	Short	Bold
Gocha	5.56±0.04	2.67±0.03	2.09±0.03	Medium	Bold
HPR-2720	5.97±0.05	2.09±0.04	2.89±0.05	Medium	Medium
HPR-2795	6.30±0.05	2.48±0.01	2.54±0.02	Medium	Medium

fibre content ranged from 0.73 (HPR-2720) to 1.95% (Jattoo). Dasgupta and Handique (2018) studied the crude fibre content in twenty Indigenous landraces of paddy, varied from 0.90 (Lalkartisali) to 1.67% (Kura binni) in pigmented red rice landraces.

Crude protein content ranged from 8.66 (Jhinjhan) to 13.89% (Gocha). The findings obtained in this study are similar to Lahkar et al., (2020), who reported protein content in traditional aromatic Joha rice varied from 6.4 (Tulsi Joha) to 17.3% (Krishna Joha). The carbohydrate content ranged from 66.30 (Jattoo) to 72.05% (Matali). Subudhi et al., (2013) also observed that carbohydrate content in aromatic rice varied from 64.6 (Pusa Sugandh-2) to 89.15% (Kala Namak). Food energy values varied significantly ranged from 334.36 (Kaluna) to 345.65kJ/100g (Gocha). Verma and Srivastav (2017) also reported food energy values for Indian rice cultivars varied from 348.79 (Govind Bhog) to 361.07 kCal/100g (Gopal Bhog).

Food energy and relationships with proximate composition: The significant negative correlation was observed between carbohydrate and protein (r = - 0.810**) at the 0.01 level, indicating an inverse relationship between carbohydrate and protein.

Additionally, a highly significant negative relationship was revealed between energy and moisture (r = - 0.877**) at the 0.01 level as well as a moderate but significant negative correlation between energy and ash (r = - 0.711*) was shown at the 0.05 level, which indicates that as the moisture and ash content of red rice varieties increases, their energy content tends to decrease. The significant negative correlation between energy and both moisture and ash were reported in several previous studies by (Verma and Srivastav 2017).

Mineral analysis: The concentrations of manganese (Mn), copper (Cu), zinc (Zn), iron (Fe), magnesium (Mg), phosphorus (P) and potassium (K) in the nine red rice varieties ranged from 24.7-40.45 mg/kg, 2.2-17.5 mg/kg 32.35-46.55 mg/kg, 83.6-402.2 mg/kg, 0.1-0.17%, 0.05-0.13% and 0.07-0.2% respectively.

Iron (Fe) was the highest micro-nutrient among all the red rice varieties followed by zinc (Zn), manganese (Mn), and copper (Cu), whereas magnesium (Mg) was the highest macro-nutrient across all the varieties, except Karad and HPR-2795, in both of these varieties, Potassium (K) was the highest macro-nutrient. The mineral content in this research is similar to or near Verma and Srivastav (2017) in six aromatic rice cultivars.

Table 4. Chemical characteristics and proximate composition of red rice

Varieties	Alkali spreading value	Gelatinization temperature	Amylose content (%)	Moisture (%)	Ash (%)	Fat (%)
Chhohartu	4.5^{d}	I (70-74° C)	25.17^{f}	11.56^{a}	2.69^{bc}	3.13^{g}
Matali	3.33^{c}	I (70-74° C)	24.94^{f}	12.77^{b}	2.63^{abc}	1.02^{a}
Jattoo	4.66^{d}	I (70-74° C)	20.55^{cd}	13.67^{d}	2.82^{c}	3.46^{h}
Karad	5.16^{d}	I (70-74° C)	18.27^{a}	12.91^{bc}	2.46^{abc}	2.76^{e}
Jhinjhan	3.5^{c}	I (70-74° C)	20.07^{ab}	13.35^{bcd}	2.27^{a}	2.62^{d}
Kaluna	2.5^{b}	H (75-79° C)	22.36^{e}	13.03^{bcd}	3.56^{d}	2.81^{b}
Gocha	4.83^{d}	I (70-74° C)	20.38^{abc}	11.95^{a}	2.35^{ab}	1.43^{f}
HPR-2720	1.66^{a}	H (75-79° C)	20.71^{d}	13.55^{cd}	3.25^{d}	2.73^{de}
HPR-2795	1.66^{a}	H (75-79° C)	20.31^{bcd}	12.98^{bcd}	2.74^{c}	2.43^{c}

H: High; I: Intermediate; L: Low
Different letters within the same column represent significant differences ($P< 0.05$).

Table 5. Proximate composition and food energy of red rice

Varieties	Crude fibre (%)	Crude protein (%)	Total carbohydrate (%)	Food energy (kJ/100g)
Chhohartu	1.17^{d}	13.08^{f}	68.33^{c}	345.00^{d}
Matali	1.12^{cd}	10.39^{b}	72.05^{e}	339.13^{bc}
Jattoo	1.95^{f}	11.78^{c}	66.30^{a}	336.19^{ab}
Karad	1.11^{cd}	13.18^{g}	67.55^{bc}	339.81^{c}
Jhinjhan	0.99^{b}	8.66^{a}	72.08^{e}	339.22^{bc}
Kaluna	1.07^{c}	13.13^{fg}	67.76^{bc}	334.36^{a}
Gocha	1.61^{e}	13.89^{h}	67.31^{b}	345.65^{d}
HPR-2720	0.73^{a}	12.16^{e}	67.64^{bc}	334.37^{a}
HPR-2795	0.79^{a}	11.90^{d}	69.13^{d}	338.82^{bc}

Different letters within the same column represent significant differences ($P < 0.05$).

Table 6. Pearson's correlation coefficient among proximate composition and food energy values in red rice

Parameters	Moisture	Ash	Fat	Fibre	Protein	Carbohydrate
Ash	0.316	-0.309	0.401	0.223	-0.810**	0.077
	(0.408)	(0.419)	(0.284)	(0.565)	(0.008)	(0.845)
Fat	-0.045	-0.241	0.221	-0.430	0.259	
	(0.909)	(0.532)	(0.568)	(0.248)	(0.500)	
Fibre	-0.105	0.294	-0.552	0.258		
	(0.788)	(0.443)	(0.124)	(0.503)		
Protein	-0.486	-0.379	0.295			
	(0.185)	(0.314)	(0.441)			
Carbohydrate	0.017	-0.711*				
	(0.965)	(0.032)				
Energy	-0.877**					
	(0.002)					

**$p < 0.01$ level (2-tailed); * $p < 0.05$ level (2-tailed)

Table 7. Mineral content of red rice (n=3)

Varieties	Micro-nutrients (mg/kg)				Macro-nutrients (%)		
	Mn	Cu	Zn	Fe	Mg	P	K
Chhohartu	40.45	13.7	33.8	259	0.15	0.11	0.13
Matali	34.65	14.2	32.35	275.95	0.1	0.05	0.08
Jattoo	34.1	14.35	42.95	247.5	0.17	0.07	0.14
Karad	24.7	12.55	44.4	83.6	0.11	0.05	0.2
Jhinjhan	29.8	15.55	32.35	402.2	0.13	0.12	0.07
Kaluna	30.85	14.6	46.55	201.75	0.14	0.07	0.12
Gocha	28.9	14.1	36.75	140.3	0.13	0.08	0.12
HPR-2720	33.2	12.2	33.8	345	0.15	0.13	0.09
HPR-2795	30.5	17.5	46.4	147.1	0.12	0.07	0.19

CONCLUSIONS

The current research provides a comprehensive assessment of the nutritional and functional attributes of red rice varieties collected from different regions of Himachal Pradesh. Red rice showed high levels of protein, ash, fat and crude fibre contributing to superior nutritional value compared to regular rice. The mineral content, particularly iron and zinc, is significantly high in varieties, this indicates the potential of red rice as a functional food that can address mineral deficiencies, especially in developing regions. The research concluded that the nutritional and functional characteristics of red rice are affected by the distinct geographical regions of Himachal Pradesh, making these varieties highly suitable for both nutritional and functional food applications. The red rice varieties of Himachal Pradesh show great potential for enhancing dietary nutrition and providing health benefits due to their rich nutrient content and bioactive compounds.

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AUTHOR'S CONTRIBUTION

Pardeep Kumar – Conceptualization, Maneesha Devi – Original draft preparation, Leena Thakur, Pushpa Guleria, Neha Guleri, Purnima Sharma – reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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Avian Community Structure in Cattle Sheds of Punjab and Haryana

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Abstract: The present study was conducted to find out whether cattle sheds could support the bird diversity. To perform the research, cattle sheds were selected from three villages each from district Ludhiana (Punjab) and Jind (Haryana), point count method was used for the collection of the data. A total of 56 avian species were recorded from the selected cattle sheds, classified into 13 orders and 32 families. Order Passeriformes was most dominant constitute 39.29% of total identified species followed by Pelecaniformes, Columbiformes, Coraciiformes, Accipitriformes, Charadriiformes, Piciformes, Bucerotiformes, Cuculiformes, Gruiformes, Psittaciformes and Galliformes, Strigiformes. From the recorded avian fauna 44 species were observed from cattle sheds of Punjab and 50 bird species were observed from the cattle sheds of Haryana with similarity index 0.679. The majority of the birds visiting cattle sheds were insectivorous (32.14%), followed by omnivorous, carnivorous, granivorous, frugivorous and nectarivorous which shows the presence of abundance of insects. However, presence of birds of different feeding guilds shows that cattle sheds provide a diverse range of food and suitable habitat to birds.

Keywords: Avian diversity, Cattle sheds, Haryana, Punjab, Species richness

Avian species are an integral part of biodiversity on this planet, their distribution depends on food availability and feeding behavior (Kler and Kumar 2015). On the Indian subcontinent, over 13% of the total world's bird diversity, or approximate 1,358 bird species are found, belonging to 26 orders and 114 families, from which 423 bird species reside in Punjab and around 520 bird species have been recorded from Haryana state (Maheswaran and Alam 2024). From the agricultural ecosystem of Punjab 213 species of birds has been recorded (Kler et al., 2022). For the assessment of the local ecosystem or regional landscapes, it is essential to have knowledge about the avian diversity and composition (Kiros et al., 2018). From the perspective of environmental monitoring, documentation of bird species assemblages in various landscapes is being prioritized (Hossain and Aditya 2016). Birds are present throughout many habitats and perform essential part in the health and balance of ecosystems (Tesfahunegny et al., 2016). Agro ecosystems, which make up 38% of the earth's land, represent one of the most productive ecosystems (Foley et al., 2011). Punjab and Haryana are widely recognized as most important agricultural states of India (Singh and Singh 2017). Availability of food and eating patterns within a given ecosystem impacts bird community structure and distribution (Sohil and Sharma 2020). In addition to food, agricultural ecosystem provides shelter and breeding sites to avian species (Kaur et al., 2017). Cattle sheds serve a dual function by providing farmers an additional source of income while serving a crucial conservation site sustaining a rich array of bird species and enriching habitat diversity (Grewal et al., 2023). Various studies have been conducted on various aspects of bird diversity in different ecosystems of Punjab (Sidhu and Kler 2017, Kaur et al., 2018) and Haryana (Chopra and Jakhar 2016, Kumar and Sahu 2019, Kumar and Sahu 2020, Singh et al., 2020), but not much work has been done regarding the diversity of birds in cattle sheds. The main objective of this study is to find the potential of cattle sheds if they can sustain the birdlife.

MATERIAL AND METHODS

Study area: Present study was conducted in villages of districts Ludhiana (Punjab) and Jind (Haryana) from April 2022 to March 2024. Cattle sheds from villages Halwara (latitude 30° 73'01.2"N, longitude 75° 64' 64.7"E), Hissowal (latitude 30° 80'70.5"N, longitude 75° 66'11.5"E), and Sudhar (latitude 30° 76'82.3"N, longitude 75° 64'89.5"E) were selected from district Ludhiana (Punjab) and named as Location I, II, and III respectively. Villages Barsola (latitude 29° 39'19.1"N, longitude 76° 23'40.8"E), Nirjan (latitude 29° 33'80.1"N, longitude 76° 37'23.1"E), and Pandu Pindara (latitude 29° 31'37.6"N, longitude 76° 36' 25.5"E) were selected from Jind (Haryana) and named as location IV, V, and VI respectively. The selected cattle sheds surrounded by agricultural fields, and a water body is present near the cattle sheds of location III and VI. Cattle sheds characterized with spacious areas, storage rooms, plastered/ unplastered walls, enough ventilation, and slip-resistant flooring bricks. Tree species recorded from the selected sheds were Kikar (*Acacia* *nilotica*), Neem (*Azadirachta indica*), Peepal (*Ficus religiosa*), and Banyan (*Ficus benghalensis*) which varies in different cattle sheds. Cattle sheds of all locations were half-shed (mostly with iron sheets) and half-open however, the cattle shed of location II is fully shed with no tree diversity. Watering and feeding areas of cattle located in shaded sections however the open section allow cattle to freely roam.

Data collection: Field observations were taken twice a month at all locations in morning (7:00-9:00 a.m.) and evening hours (5:00-7:00 p.m.). Point count method was used for the observation of birds visiting cattle sheds by naked eyes or with the help of binoculars. Photographs of observed species were taken by using Canon EOS 1200D to identify bird species. Identification of the birds were done on the basis of their characters as described by Grimmett et al. (2013). List of the recorded bird's species was prepared using the checklist prepared by Praveen et al. (2016). IUCN status of the birds recorded by using IUCN Red List of Species (IUCN 2023). Feeding guilds of birds were classified by using available literature and direct observation during field study (Kumar et al., 2019).

Data analysis: From the collected data species richness (total bird species observed from a location), relative abundance, species diversity by Shannon-Wiener Index (H´) (Sekhon et al., 2024), and Jaccard's similarity index (Cj) (Kumar and Sahu 2019) were calculated by using appropriate formulas in MS excel 2013. Kruskal Wallis and Mann-Whitney U test were used to analyze the data statistically using SPSS version 16 computer software.

RESULTS AND DISCUSSION

During the present study period a total of 56 birds were recorded from cattle sheds of the selected villages of Punjab and Haryana which belongs to 13 orders and 32 families (Table 1). Order Passeriformes was most dominant constitute 39.29% of total identified species followed by Pelecaniformes, Columbiformes, Coraciiformes , Accipitriformes, Charadriiformes, Piciformes (Bucerotiformes, Cuculiformes, Gruiformes, Psittaciformes and Galliformes, Strigiformes (1.79%) (Fig. 1).

From the villages of Punjab 44 bird species were recorded, belonging to 11 orders and 29 families with species richness of 36, 26, and 29 at locations I, II, and III respectively. Shannon-Wiener Index was highest at Location I (2.15), followed by Location II (1.86) and Location III (1.71). Fifty bird's species were recorded from the villages of Haryana belonging to 13 orders and 29 families with species richness of 33, 29, and 38 at Location IV, V, and VI respectively. Shannon-Wiener Index was highest at Location V (1.93), followed by Location VI and Location IV (Table 2). Jaccard's similarity index (Cj) shows similarity of 0.679 in Punjab and Haryana. Singh and Laura (2012) stated that avian diversity is correlated directly with plant diversity because plants provide birds a place for nesting, and reproduction and also food to eat. Present study showed variation in bird species richness in different location being highest in location VI (38), followed by I, IV, III, V and location II. The highest species richness was due to the good structure and vegetation of cattle sheds at Location VI and I,

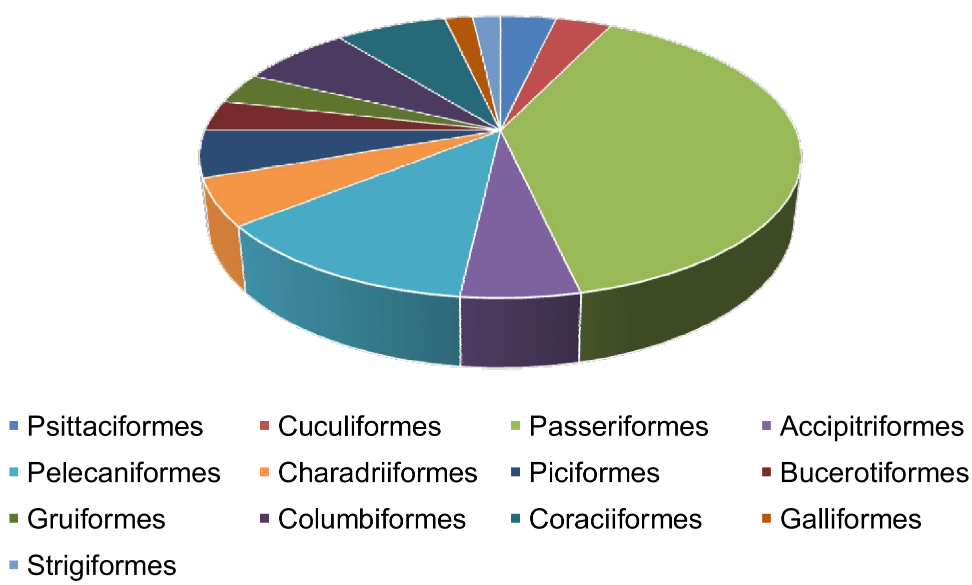


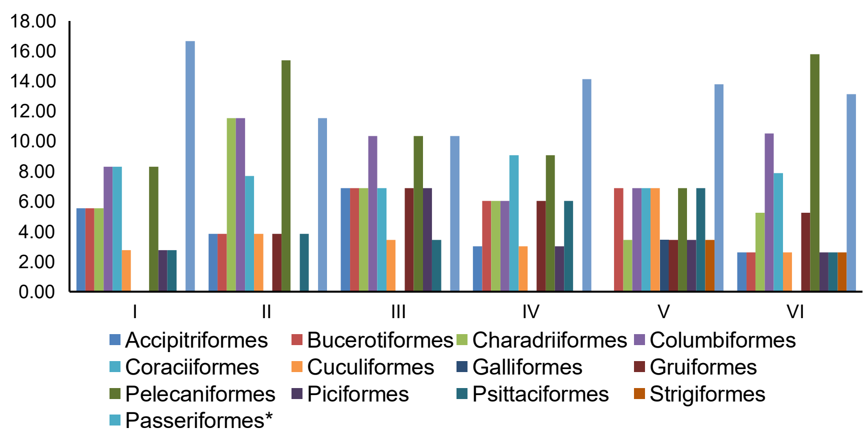
Fig. 1. Percent composition of different bird orders in cattle sheds of Punjab and Haryana

Table 1. Bird species from cattle sheds of Punjab and Haryana

Species	Scientific name	Order	Family	IUCN status	Feeding guild
Alexandrine Parakeet	*Psittacula eupatria*	Psittaciformes	Psittaculidae	NT	F
Asian Koel	*Eudynamys scolopaceus*	Cuculiformes	Cuculidae	LC	O
Asian Pied Starling	*Gracupica contra*	Passeriformes	Sturnidae	LC	O
Bank Myna	*Acridotheres ginginianus*	Passeriformes	Sturnidae	LC	I
Baya Weaver	*Ploceus philippinus*	Passeriformes	Ploceidae	LC	G
Black Drongo	*Dicrurus macrocercus*	Passeriformes	Dicruridae	LC	I
Black Kite	*Milvus migrans*	Accipitriformes	Accipitridae	LC	O
Black-headed Ibis	*Threskiornis melanocephalus*	Pelecaniformes	Threskiornithidae	NT	C
Black-winged Kite	*Elanus caeruleus*	Accipitriformes	Accipitridae	LC	C
Black-winged Stilt	*Himantopus*	Charadriiformes	Recurvirostridae	LC	O
Brahminy Starling	*Sturnia pagodarum*	Passeriformes	Sturnidae	LC	I
Brown Rock Chat	*Oenanthe fusca*	Passeriformes	Muscicapidae	LC	I
Brown-headed Barbet	*Psilopogon zeylanicus*	Piciformes	Ramphastidae	LC	F
Cattle Egret	*Bubulcus ibis*	Pelecaniformes	Ardeidae	LC	C
Common Hoopoe	*Upupa epops*	Bucerotiformes	Upupidae	LC	I
Common Moorhen	*Gallinula chloropus*	Gruiformes	Rallidae	LC	O
Common Myna	*Acridotheres tristis*	Passeriformes	Sturnidae	LC	O
Common Sandpiper	*Actitis hypoleucos*	Charadriiformes	Scolopacidae	LC	C
Common Tailorbird	*Orthotomus sutorius*	Passeriformes	Cisticolidae	LC	I
Coppersmith Barbet	*Psilopogon haemacephalus*	Piciformes	Ramphastidae	LC	F
Eurasian Collared Dove	*Streptopelia decaocto*	Columbiformes	Columbidae	LC	G
Great Egret	*Ardea alba*	Pelecaniformes	Ardeidae	LC	C
Greater Coucal	*Centropus sinensis*	Cuculiformes	Cuculidae	LC	O
Green Bee-eater	*Merops orientalis*	Coraciiformes	Meropidae	LC	I
House Crow	*Corvus splendens*	Passeriformes	Corvidae	LC	O
House Sparrow	*Passer domesticus*	Passeriformes	Passeridae	LC	G
Indian Black Ibis	*Pseudibis papillosa*	Pelecaniformes	Threskiornithidae	LC	C
Indian Grey Hornbill	*Ocyceros birostris*	Bucerotiformes	Bucerotidae	LC	O
Indian Peafowl	*Pavo cristatus*	Galliformes	Phasianidae	LC	O
Indian Pond Heron	*Ardeola grayii*	Pelecaniformes	Ardeidae	LC	C
Indian Robin	*Saxicoloides fulicatus*	Passeriformes	Muscicapidae	LC	I
Indian Roller	Coracias benghalensis	Coraciiformes	Coraciidae	LC	I
Indian Silverbill	*Euodice malabarica*	Passeriformes	Estrildidae	LC	G
Jungle Babbler	*Turdoides striata*	Passeriformes	Leiothrichidae	LC	O
Laughing Dove	*Streptopelia senegalensis*	Columbiformes	Columbidae	LC	G
Lesser Golden-backed Wood pecker	*Dinopium benghalense*	Piciformes	Picidae	LC	I
Little Cormorant	*Microcarbo niger*	Pelecaniformes	Ardeidae	LC	C
Little Egret	*Egretta garzetta*	Pelecaniformes	Ardeidae	LC	C
Oriental Magpie Robin	*Copsychus saularis*	Passeriformes	Muscicapidae	LC	I
Pied Kingfisher	*Ceryle rudis*	Coraciiformes	Alcedinidae	LC	C
Plain Prinia	*Prinia inornata*	Passeriformes	Cisticolidae	LC	I
Purple Sunbird	*Cinnyris asiaticus*	Passeriformes	Nectariniidae	LC	N
Red-vented Bulbul	*Pycnonotus cafer*	Passeriformes	Pycnonotidae	LC	G
Red-wattled Lapwing	*Vanellus indicus*	Charadriiformes	Charadriidae	LC	O
Rock Pigeon	*Columba livia*	Columbiformes	Columbidae	LC	G
Rose-ringed Parakeet	*Psittacula krameri*	Psittaciformes	Psittaculidae	LC	F
Rufous Treepie	*Dendrocitta vagabunda*	Passeriformes	Corvidae	LC	I
Shikra	*Accipiter badius*	Accipitriformes	Accipitridae	LC	C
Spotted Owlet	*Athene brama*	Strigiformes	Strigidae	LC	C
Western Yellow Wagtail	*Motacilla flava*	Passeriformes	Motacillidae	LC	I
White Wagtail	*Motacilla alba*	Passeriformes	Motacillidae	LC	I
White-breasted Waterhen	*Amaurornis phoenicurus*	Gruiformes	Rallidae	LC	O
White-browed Wagtail	*Motacilla maderaspatensis*	Passeriformes	Motacillidae	LC	I
White-throated Kingfisher	*Halcyon smyrnensis*	Coraciiformes	Alcedinidae	LC	I
Wire-tailed Swallow	*Hirundo smithii*	Passeriformes	Hirundinidae	LC	I
Yellow-legged Green Pigeon	*Treron phoenicopterus*	Columbiformes	Columbidae	LC	G

NT (Near Threatened), LC (Least concern), I (Insectivorous), O (Omnivorous), C (Carnivorous), G (Granivorous), F (Frugivorous), N (Nectarivorous) which provide suitable space to birds. Birds are also extremely sensitive to invasions from humans and the abundance of many bird species decreases in proximity of human settlements. The disturbances caused by human's activities for cattle management in cattle sheds can reduce the avian diversity, especially in fully shed system where space is more confined and disturb the bird species. The fully shed structure of cattle shed at Location II can be a reason for low species richness and diversity. Kruskal Wallis test shows a significant difference in species diversity at different locations of Punjab and Haryana. Mann-Whitney U test at location I differs significantly from locations II, III, V, and VI, and Location III differs significantly to locations IV and V (Table 3). The significant difference between the different locations is may be due to the different vegetation, structure and surrounding environments of these cattle sheds. Tree diversity and agricultural fields near the cattle sheds are the main components influencing the avian diversity.

Avian species recorded from all the locations belong to orders Columbiformes, Bucerotiformes, Charadriiformes, Coraciiformes, Cuculiformes, Pelecaniformes, Passeriformes, and Psittaciformes. Order Accipitriformes observed at locations I, II, III, IV, and VI, Gruiformes observed at locations II, III, IV, V and VI, Piciformes observed at locations I, III, IV, V and VI, Strigiformes observed at location V and VI, Galliformes observed only at location V (Fig. 2). Order Passeriformes was dominant at all locations with the abundance (%) varying from 31.03 to 50.00 in selected locations. Strigiformes (Spotted Owlet with relative abundance of 0.57% and 0.12% at location V and VI respectively) and Galliformes (Indian Peafowl with relative abundance 6.33% at location V) were the least representing bird orders with one species each. Chopra et al. (2012) recorded Passeriformes as dominant bird order and



*Value for order Passeriformes is three times more than the value of other orders

Fig. 2. Representation of different bird orders observed in cattle sheds of Punjab and Haryana

Table 2. Community characteristics of bird species recorded from the cattle sheds of Punjab and Haryana during April 2022-March 2024

Community characteristics	Locations					
	I	II	III	IV	V	VI
Species richness	36	26	29	33	29	38
Species diversity	2.15	1.86	1.71	1.85	1.93	1.89
Species evenness	0.87	0.86	0.81	0.81	0.86	0.81

Table 3. Mann-Whitney U table for species diversity at selected locations

Locations	II	III	IV	V	VI
I	0.002	0.000	0.101	0.012	0.014
II	-	0.143	0.242	0.514	0.713
III	-	-	0.028	0.017	0.089
IV	-	-	-	0.514	0.551
V	-	-	-	-	0.799

Podicipediformes and Strigiformes as the least representing bird orders in Sultanpur National Park Gurgaon, Haryana. Passeriformes was most dominant order and this outcome aligns with the findings of majority of the studies that has been conducted on different aspects of avian diversity mainly in agricultural and pond ecosystems of Punjab and Haryana. Agricultural landscapes of district Panipat are also dominated by Passeriformes (Kumar and Sahu 2019). Sekhon et al. (2023) found order Passeriformes as most abundant during their study in village ponds of Punjab state with 32 families.

Grewal et al. (2023) recorded 26 bird species in the cattle sheds from the villages of Ludhiana. The structural plan of the cattle shed provides a safe cover to the bird species, because of this cattle sheds has the ability to conserve bird species. The current study demonstrates that the existence of birds belongs to six different feeding guilds, Insectivorous (32.14%), followed by Omnivorous, Carnivorous, Granivorous, Frugivorous, and Nectarivorous (1.79%), which shows that cattle sheds offer a variety of food resources to birds as well as a good place and space to feed.

CONCLUSION

The cattle sheds has the potential to support a diversity of bird species. This is because of its structural plan, tree diversity and presence of different types of food for birds. The bird diversity in rural ecosystem can be conserve by improving structural plan and habitat quality of cattle sheds.

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Evaluation of Acute and Chronic Toxic Effect of *Calotropis procera* Leaf Extract on *Bandicota bengalensis*

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Abstract: *Calotropis procera* has nephroprotective and hepatoprotective properties. Its hay is used for increasing the meat quality. Besides being medicinal plants, also have suggested its pesticidal effects. For the present investigation, three types of baits were prepared (two from dry leaf powder alcoholic extract and one from fresh leaves juice) and given in bi-choice condition to field rat, *Bandicota bengalensis* for three days to observe its acute toxicity. Treatment period was also extended to 30 days to see the chronic toxic effect on kidney through histological studies and biochemical analysis. *C. procera* dry leaf powder alcoholic extracts and fresh leaves juice based baits had no acute toxicity against *B. bengalensis* when given under bi-choice condition for three days. Chronic toxicity test also revealed no histopathological alteration in kidneys. Total soluble protein content of kidney of treated rats decreased significantly compared to untreated rats. However, a non-significant biphasic trend was recorded in the antioxidant enzymatic activity in treated groups in comparison to untreated group. Thus *C. procera* leaf extracts based baits have neither acute nor chronic toxicity effects. Thus, these cannot be used as pesticide against rodents.

Keywords: *Calotropis procera*, *Bandicota bengalensis*, Histological studies, Biochemical analysis

The herbal medicines hold prominent place right from ancient period in Indian Ayurveda. *Calotropis procera* is one of the most valuable medicinal plant. It is also found in other countries like Malaysia, Sri Lanka, Bangladesh, Pakistan, Bhutan, Nepal and belongs to family Ascalpidaceae and has both therapeutic and pesticidal properties. It is a xerophytic plant found in tropical, subtropical areas and also extended into temperate regions (Nasser et al., 2012). Morphologically, it has simple slightly leathery leaves with simple stem and white and purple coloured flowers (Joseph et al., 2013). It is a laticiferous plant and grows up to 2.5 m (Moustafa and Sarah 2017). Arial parts of the plants contain a sticky substance or a milk sap called latex.

All parts of the *C. procera* such as leaves, roots, flower, bark, latex are used for various purposes and for the treatment of various diseases all over the world since antiquity. Total 547 types of formulations are formed from *Calotropis procera*, which is treating around 58 diseases (Kale et al., 2022). These plant parts are used for the treatment of toothache, earache, fungal infection like ringworm, stings, rheumatism, to relieve pain, epilepsy, skin infections, leprosy, diarrhoea, malaria, ulcers, mental disorders, spleen, liver problems, wound-healing, as analgesic, pro-coagulant (Abeysinghe 2018), and also having anti cancerous properties (Chandekar et al., 2020). It was also used as nephroprotective and hepatoprotective agent. In Andhra Pradesh, Bagata tribes of Vishakhapatnam district use dried roots and latex as an antidote for poisonous snake biting (Seema and Anitha 2015). Phytochemicals such as carbohydrate, tannins, cardiac glycoside, flavonoids, terpenoid, saponin and saponin glycoside are present in *C. procera* and have therapeutic values (William et al., 2015). Hay from *C. procera* was also reported to be a good animal food because it has high protein content and is highly digestible (Madruga et al., 2008). Besides being medicinal plant and its use in increasing the meat quality, several authors also have suggested its toxic and harmful effects. In rats and sheeps cardiotoxic and hepatotoxic effects were observed (Lima et al., 2011). In mice, LD_{50} value with dry latex was 3 g/kg body weight. However, in rodents, no lethal effect was recorded at 165 to 830 mg/kg body weight (Dewan et al., 2000). *C. procera* is being widely used both for its medicinal and poisonous effects, depending upon the dose and mode of use. Even an acute poison act as a drug if it is taken in prescribed manner. Earlier studies reported the beneficial medicinal value of this plant at low doses and toxicity at high doses (William et al., 2015). During present study, baits using high doses of *C. procera* extract were prepared to utilize the toxic property of *C. procera* against rodents pests.

MATERIAL AND METHODS

Experimental site and preparation of *C. procera* extracts and juices: This study was conducted at Rodents Research Laboratory and Animal House of Punjab Agricultural University, Ludhiana, Punjab, India. *C. procera* leaves were collected from barren land surrounds Ladhowal Seed Farm, Punjab Agricultural University, Ludhiana. Both stock *C. procera* dry leaf powder alcoholic extract (CLAE-60ml) and fresh leaf juice (FLJ-50ml) were prepared using 100g *C. procera* dry leaves powder and fresh leaves respectively. The known quantity of stock *C. procera* leaf alcoholic extract was mixed in known amount of WSO (Wheat:Sugar:Oil; 96:2:2) based plain bait for the preparation of treated baits 1 and 2. Fresh leaf juice was extracted by grinding fresh leaves in grinder, which were then hand squeezed to collect the fresh leaves juice, mixed in WSO bait to prepare treated bait 3.

Preparation of treated baits: Treated bait 1 was prepared by mixing 20 ml of CLAE in 100 g WSO bait to prepare 33.3% dried leaf powder treated WSO bait. Treated bait 2 was prepared by mixing 30 ml of CLAE in 100 g WSO bait to prepare 50% dried leaf powder treated WSO bait. Treated bait 3 was prepared by mixing 50 ml of fresh leaves juice (from 100 g fresh leaves) in 100g WSO bait.

Collection and maintenance of animals: The lesser bandicoot rats, *Bandicota bengalensis* were trapped from grocery shops and were divided into 4 groups (n = 3 of each group). Before the start of the experiment, rats were acclimatized. For acclimatization, rats were kept in individual laboratory cages of size 36 × 23 × 23 cm with food and water provided *ad libitum* for 10-15 days. Cracked wheat, powdered sugar, and vegetable oil (WSO bait) were mixed in a ratio of 96:2:2 to prepare the bait for rats. Approval for the usage of animals was taken from the Institutional Animal Ethics Committee (IAEC), Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (vide memo no. GADVASU/2019/IAEC/49/04 dated: 18, April 2019, during XLIX Meeting of IAEC). Guidelines on the regulation of scientific experiments on animals were followed (Pandey and Sharma 2011). Proper hygienic conditions were maintained. Plastic trays were kept under each laboratory cage for collection and disposal of animal faeces and urine.

Acute and chronic toxicity: Group I rats were fed on plain WSO bait. Rats of groups II, III and IV were fed on treated baits 1, 2 and 3 respectively for three days in bi-choice to record their consumption and acute toxicity. After that same groups of rats were fed on *C. procera* treated baits under bi-choice conditions for 30 days to determine the chronic toxic effect of the same baits on kidneys.

Histomorphological and biochemical studies: Kidneys were collected from both treated and untreated rats after 30 days of treatment period and a piece of kidney was fixed in 10% NBF (natural buffer formalin) and processed as per standard method for histological studies (Humason 1966). Bowman's capsule (BC), proximal convoluted tubules (PCT), distal convoluted tubules (DCT), ascending limbs (AL), descending limbs (DL) in the transverse sections of kidneys were identified (Victor, 2017). Number of BC, PCT, DCT, AL and DL in the transverse section of kidneys of different groups of rats was determined. The known weight of kidney was also homogenized in phosphate buffer saline and then centrifuged at 3000 r.p.m. for 10 min. The supernatant was used for quantative estimations of total soluble protein (Lowry et al., 1951) and to determine the specific activity of antioxidants such as superoxide dismutase (Marklund and Marklund 1974), glutathione peroxidase (Hafeman et al., 1984), glutathione-S-transferase (Habig et al., 1974) and glutathione reductase (Carlberg and Mannervik 1985).

Statistical analysis: SPSS 16.0 and SAS 9.3 software were used for statistical analysis.

RESULTS AND DISCUSSION

Acute toxicity: The pre-treatment consumption signified that all the rats were healthy and can be used for treatment. The overall consumption of all the three treated baits was significantly less than the untreated bait indicating that all the treated baits have less palatability as compared to the untreated bait. Percent acceptance (%) of treated baits was non-significantly different among treated groups with ranging from 33.36% (bait 3) to 56.03% (bait 1). The bait 1 having minimum dose of CLAE has maximum palatability and percent acceptance was significantly higher than bait 3. Active ingredient (CLAE and FLJ) consumed per day was also non-significantly different among groups with values ranging from 1.22 g (bait 3) to 1.77 g/100 g b. wt. (bait 1) indicating consumption of active ingredient was maximum with treated bait 1. Although consumption of active ingredients (*C. procera* leaf) with three treated baits/day ranged from 1.22 to 1.77 g/100 g b. wt. with percent acceptance in comparison to plain bait being 33.36 to 56.03% for a period of three days but mortality was nil with all the three treated baits indicating *C. procera* dry leaf extract based baits and fresh leaf juice based bait had no lethal effects (Table 1). Earlier acute toxicity test conducted in albino rats reported that LD_{50} value of *C. procera* leaf extract is 774 mg/kg b. wt. (William et al., 2015). No toxicity observed during present study might be due to the less bioavailability of secondary metabolites when mixed in bait because of reduction in absorption of dietary fats, protein and fibres (Bushra et al., 2011). The average daily consumption of all the treated baits was lowest on first day of treatment period. However with increase in duration of their exposure, rats developed habituation to all the three treated baits and consumption of treated baits increased with time. However, there was a non-significant difference in the consumption of treated bait among days. On day one, initially for about 2 hours, rats were inactive and showed neophobic behaviour towards the treated baits. But after that rats were normal and active and consumed treated bait along with plain bait; although percent acceptance of all the treated baits was less than the plain bait. Therefore acute toxicity test of baits treated with alcoholic extract of *C. procera* dry leaf powder and fresh leaf juice on *B. bengalensis* revealed that all the three treated baits containing 33.3 to 50% dry leaf powder and fresh leaves have no lethal effect on rats. Therefore, these baits in present form can't be used as rodenticides against dominating crop field rats, lesser bandicoot rats. There is a need to develop a formulation using CLAE to increase the bioavailability of treated bait.

C. procera products contain natural secondary metabolites called glycosides. These glycosides are toxic and poisonous in nature. Glycosides present in the leaves of *C. procera* include calotropin, calotopagenin, calotoxin, calactin, uscharin, mudarine (bitter yellow acid) and resin. *C. procera* is well known for its pesticidal properties. It has been successfully used against many pests and has been reported as one of the most promising plants which have potential as alternative to chemical pesticides (Begum et al., 2013,Eisa and Yassin 2016,). It was hypothesized that it can be applied in fields against rodents as bait. However during present investigation, toxic symptoms after ingestion of treated baits 1 - 3 were not recorded. Therefore these baits in present form cannot be used as rodenticide against predominant rodent pest, *B. bengalensis*. Earlier study also reported that administration of single high dose i.e. 3 g/kg ethanolic extract of *C. procera* did not cause any mortality or visible toxicity symptoms. However, treatment for long period (90 days) caused significant mortality (Ahmed et al., 2005). Acute toxic (behavioural and neurological responses) effects were however not recorded in albino rats after administration of up to 2500 g/kg body weight of latex, ethanolic, chloroform and aqueous leaves extract of *C. procera* for 7 days (Ismaiel and Alrheam 2015). Death and acute toxicity was also not observed in male Wistar rats after 48 hours when fresh latex was injected intra-peritoneally at doses ranges from 0.1 to 0.6 ml of latex/kg of b. wt. (Lima et al., 2011). No mortality was recorded in mice with dry latex of *C. procera* administered orally at doses ranging from 165-830 mg/kg (Dewan et al., 2000).

However, an earlier study reported 20- 80.5% mortality in Norway rats administered with 21.5- 215 mg /kg of *C. procera* leaf extract respectively (Eisa and Yassin 2016). For male albino rats, LD_{50} value was 95.52 mg/kg (El-Shafey et al., 2011) while for Wistar rats, LD_{50} value was 993 mg/kg (Manivannam et al., 2011). Seema and Anitha (2015) also reported that stem and roots of *C. procera* is toxic than *C. gigantica* and even more hazardous than the cobra's venom. Latex is irritant and having neurotoxic and anticholinergic properties which cause toxicity and sometime death. The fatal or death period varies from 30 min to 8 hr. (Seema and Anitha 2015). However, during present investigation mortality was not recorded even after consumption of very high dose of *C. procera* dry leaf extract and fresh juice. Toxicity of dry leaf extract and fresh leaf juice are reduced when mixed in bait. Therefore, there is a need to develop a formulation/ bait with enhanced bioavailability of secondary metabolites of *C. procera* leaf extract.

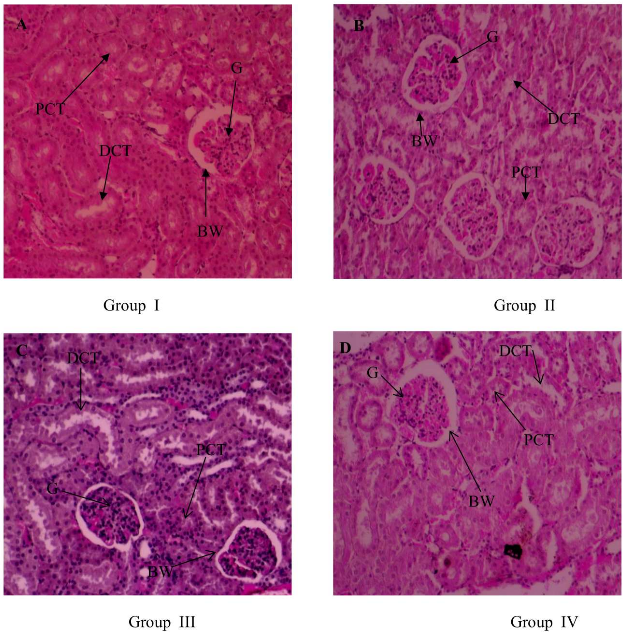
Effect on histomorphology of kidney: A chronic toxicity test was also conducted for a period of 30 days to access the effect of same *C. procera* dry leaf powder and fresh leaf juice based treated baits on histomorphology and activity of antioxidant enzymes in kidneys of treated rats. During treatment period of 30 days, percent acceptance of different treated baits/day ranged from 44.07 to 69.30%. There was no effect of treatment on histomorphology of kidneys. No significant pathological effect of treatment was observed on the numbers and histoarchitecture of Bowman's capsule, proximal convoluted tubules, distal convoluted tubules in cortical region and loop of henle's, descending limb and ascending limb in medullary region of kidney (Table 2, Fig. 1). These results signified that *C. procera* did not cause any

Table 1. Consumption (g/100 g b wt.), percent acceptance and active ingredient consumed per day of *C. procera* leaf based treated baits by *B. bengalensis*

Treatments (n=3)	Body weight (g)	Consumption of baits (g/100 g b wt./day)			Acceptance of treated bait (%)	Active ingredient consumed per day (g/100 g b wt.)	Mortality (%)
		Pre-treatment period	Treatment period				
			Plain	Treated			
Treated bait 1 (CLAE : 33.3% DLP)	270 ^{a}	10.50 ^{a}	6.31 ^{b}	3.64 ± 0.13^{b}	56.03 ± 0.95^{a}	1.77 ± 0.03^{a}	Nil
Treated bait 2 (CLAE:50% DLP)	273 ^{a}	10.53 ^{a}	7.52 ± 0.57^{ab}	3.27 ± 0.30^{ab}	44.60 ± 6.33^{ab}	1.64 ± 0.15^{a}	Nil
Treated bait 3 (FLJ: 50%FL)	258 ^{a}	10.51 ^{a}	8.05 ± 0.31^{a}	2.68 ± 0.42^{a}	33.36 ± 5.43^{b}	1.22 ± 0.24^{a}	Nil

Different superscripts indicates significant difference ($p \leq 0.05$) in consumption between plain and treated baits along the rows as well as among baits along the columns
CLAE: *Calotropis procera* leaf alcoholic extract, DLP: Dry Leaf powder; FLJ: Fresh leaf juice, FL: Fresh leaves

chronic toxic effect on kidney of rats. However, earlier study reported that administration of ethanolic leaf extract (1/20 of LD_{50}) of *C. procera* for 4 weeks begins reduction in luminal space of Bowman's capsule and this space was completely vanished with increase in concentration of *C. procera* ethanolic leaf extract (Fahim et al., 2016). Level of urea, uric acid and serum creatinine were increased with the consumption of *C. procera* latex or ethanolic leaf extract which interfered in renal activities or damaged tubular epithelial cells (Ahmad et al., 2014). Ethanolic extract (200-400 mg/kg) of *C. procera* roots was also documented to have hepatoprotective and nephroprotective properties (Prakash et al., 2011). The aqueous extract of *C. procera* flower have nephroprotective effect on rabbits when co-ingested with gentamicin (Javed et al., 2014).



BW: Bowman's capsule, G: Glomerulus, PCT: Proximal convoluted tubules, DCT: Distal convoluted tubules, DL: Descending limb, AL: Ascending limb
Group 1: Untreated rats; Group II: Rats fed on treated bait 1; Group III: Rats fed on treated bait 2; Group IV: Rats fed on treated bait 3

Fig. 1. T.S of kidney of different groups of rats (100X, H & E)

Effect on anti-oxidants: The total soluble protein content of kidney of group II treated rats decreased significantly as compared to untreated rats. However activities of anti-oxidants were non-significantly different in all the treated groups as compared to the untreated group. Specific activity of superoxide dismutase, glutathione reductase and glutathione-S-transferase decreased slightly in treated groups in comparison to untreated group. However, the specific activity of glutathione peroxidase remained same in untreated and treated groups (Table 3). Earlier studies also reported that the dichloromethane, hexane and ethyl acetate crude extracts of *C. procera* are toxic at 250 and 500 µg/mL, on the other hand, methanol and aqueous extracts were less toxic even at > 2000 µg/mL but the lower concentrations

Table 2. Effect of *C. procera* leaf based treated baits on cortex and medullary region of kidney of *B. bengalensis*

Treatments	Number/mm^2 in cortical region			Number/mm^2 in medullary region	
	Bowman's capsule	PCT	DCT	DL	AL
Group I (Untreated bait)	2.01 ± 0.03^a	5.18 ± 0.12^a	1.37 ± 0.15^a	5.45 ± 0.06^a	5.40 ± 0.08^a
Group II (Treated bait 1)	2.0 ± 0.04^a	5.11 ± 0.08^a	1.36 ± 0.07^a	5.35 ± 0.05^a	5.47 ± 0.02^a
Group III(Treated bait 2)	2.00 ± 0.03^a	5.14 ± 0.06^a	1.38 ± 0.11^a	5.39 ± 0.09^a	5.48 ± 0.03^a
Group IV (Treated bait 3)	1.97 ± 0.02^a	5.17 ± 0.07^a	1.27 ± 0.08^a	5.41 ± 0.04^a	5.52 ± 0.02^a

Similar superscripts indicate non- significant difference ($p \leq 0.05$) along the columns
Untreated bait: WSO mixed plain bait
Treated bait 1: *C. procera* alcoholic leaf extract (CLAE) = 33.3% Dry Leaf Powder (DLP) in WSO bait
Treated bait 2: *C. procera* alcoholic leaf extract (CLAE) = 50% Dry Leaf Powder (DLP) in WSO bait.
Treated bait 3: Fresh leaf juice (FLJ) = 50% Fresh leaves (FL) in WSO bait.
PCT: Proximal convoluted tubules, DCT: Distal convoluted tubules, DL: Descending limb, AL: Ascending limb

Table 3. Effect of *C. procera* leaf extract based treated baits on total protein content and specific activity of different antioxidants

Treatments	Soluble protein content (mg/g)	SOD (U/mg protein)	GP_x (U/mg protein)	GR (µ moles of NADPH conjugate / min/ mg protein)	GST (µ moles of GSH-CDNB conjugate formed / min /mg protein)
Group I (Untreated bait)	11.28 ± 0.07^c	25.46 ± 4.77^a	0.62 ± 0.038^a	0.158 ± 0.02^a	0.361± 0.064^a
Group II (Treated bait 1	10.70 ± 0.03^b	22.09 ± 2.10^a	0.60 ± 0.045^a	0.069 ± 0.01^a	0.346 ± 0.015^a
Group III (Treated bait 2)	9.67 ± 0.15^a	20.42 ± 0.36^a	0.61 ± 0.040^a	0.081 ± 0.027^a	0.303 ± 0.040^a
Group IV (Treated bait 3)	10.11 ± 0.18^a	23.00 ±3.25^a	0.63 ± 0.069^a	0.115 ± 0.039^a	0.327 ± 0.079^a

Different superscripts indicate significant difference ($p \leq 0.05$) along the columns
Untreated bait: WSO mixed plain bait
SOD: Superoxide dismutase, GP_x: Glutathione peroxidase, GR: Glutathione reductase, GST: Glutathione-S-transferase

(100-12.5 µg/mL) had no toxic effects and did not cause any morphological changes in cells. Calo-protein (1000 µg/mL) from stem bark of *C. procera* also did not produce any toxic or harmful effect on skin cells (Samy and Chow 2012). *C. procera* have beneficial medicinal value at low doses (118 mg/kg b. wt.) and toxicity at high doses (774 mg/kg b. wt.). Therefore, recommended that shouldn't be used for medicinal purposes at higher concentration (William et al., 2015). However during present investigation, no toxicity was recorded even at high dose. *C. procera* leaf extracts have neither acute nor chronic toxic effects when mixed in bait. Earlier studies reported that it can be safely used for its medicinal properties as well as can be fed to domestic animals for increasing meat quality but cannot be used as rodenticide (Eisa and Yassin 2016).

CONCLUSIONS

C. procera leaf extracts have no (acute and chronic) toxic effects when mixed in bait against *B. bengalensis* and it shows no lesions and inflammation on kidney. So it can be used as medicinal herbs and as fodder for domestic animals but cannot be used as rodenticides.

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Impact of Chickpea, Soybean and Wheat Straw on Cultivation of Oyster Mushroom (*P. membranaceus*)

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***Abstract*:** This experiment aimed to evaluate the use of soybeanstraw (SS) and chickpea straw (CS) with wheat straw (WS) in different rations like 100%: 0%, 75%: 25%, 50%: 50%, 25%: 75% and 0: 100% to grow *P. membranaceus* as an edible mushroom. Among all aspects, WS (control) was the best substrate with yield (902.10 gm) and biological efficiency (90.21%) followed by SS 75% + 25 % WS (865.08g and 86.50%) and the lowest was from CS 100% (764.30 g and 76.43%).The study corroborates that WS is one of the best substrates for oyster mushroom cultivation, while combinations with SS can enhance certain fruiting characteristics. However, CS appears less effective for growth and yield, potentially due to its slower decomposition and lower nutrient availability. Future studies could explore additional supplementation of CS to improve its performance.

***Keywords*:** Mushroom cultivation, *P. membranaceus*, WS, Chickpea straw, Biological efficiency

Oyster mushrooms (*Pleurotus* spp.) are among the most widely cultivated edible mushrooms globally due to their high nutritional value, medicinal properties and ability to grow on various lignocellulosic substrates. Traditionally, substrates such as rice straw, WS and maize have been employed for mushroom cultivation. However, the search for alternative, cost-effective substrates that can enhance yield and promote sustainable agricultural practices has intensified recently (Koutrotsios et al., 2014). Chickpea (*Cicer arietinum*) and soybean (*Glycine max*) straws are by-products of legume cultivation that are rich in lignin, cellulose and hemicelluloses-components crucial for the growth of oyster mushrooms. Utilizing these agricultural residues not only adds value to otherwise underutilized by-products but also contributes to environmental sustainability by reducing agricultural waste (Patel et al., 2012). *Pleurotus membranaceus* is widely consumed globally due to its taste, flavor, high nutritional content and medicinal properties. *P. membranaceus* has a pileus diameter of 3-10 cm, dimidiate to flabelliform, with a skinny point of attachment and attenuate base; the floor is white (after drying it seems cinnamon brown), coarsely striate and the margins are lobed and irregular. Stripe is usually absent; then again it might show up in occasion

In addition, *P. membranaceus* may offer potential advantages when integrated into the cultivation substrate. If *P. membranaceus* is a plant-derived component, it might provide additional nutrients or bioactive compounds that enhance fungal growth and yield. If it refers to a specific strain of *Pleurotus*, it could offer better adaptation to the substrates and environmental conditions, improving overall productivity (Owaid et al., 2015, Kausar et al., 2020). The study focuses on optimizing substrate formulations to maximize yield, improve mushroom quality and assess the added benefits of *P. membranaceus* in the cultivation process. By exploring these novel substrate combinations, this study contributes to the development of more sustainable and efficient mushroom production practices, while also providing an innovative use for agricultural residues.

MATERIAL AND METHODS

These steps are used in oyster mushroom production procedures pure culture preparation**,** straw preparation, spawn production, growing and harvesting at maturity stages at different intervals.

Location and treatments: The present finding was carried out at the Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.)located at 23.09° N latitude and 79.58° E longitude at an altitude of 411.78 meters above the sea level The single factor experiment consisted of five treatments, i.e., different types of substrates: SS (RS), CS (CS) mixed with WS (WS) in different combination.

Pure culture preparation: The pure culture was isolated from the fruiting body of *P. membranaceus* in the Mushroom Research Laboratory of Jabalpur and multiplied on potato dextrose agar medium for 7 days before being kept in test tubes with potato dextrose agar. After being sterilized for 30 minutes at 121°C and 1.5 *p.s.i*, clean test tubes were allowed to harden in a slant position. To create pure culture, a small amount of soft tissue (from the original *P. membranaceus* culture) was aseptically transferred to individual PDA slants at 20±2°C. Once the mycelium had completely colonized the agar media, the culture was utilized to prepare the spawn.

Spawn preparation: Wheat (*Triticum aestivum*) grain was used in the spawn preparation process.Wheat grain was partially boiled for 20–25 minutes, rinsed and left to cool to room temperature. The pH of the grain was adjusted to 9.0 and 1% CaCo3 (calcium carbonate) was added as a food supplement (Romero 2007). The boiled grain was filled in glass bottles into two-thirds portions, sealed and pasteurized for one hour at 121 °C and 1.5 *p.s.i* to sterilize them. Sterilized glass bottles were aseptically inoculated with mycelia culture bits (5mm). The bottles were kept at 20±2 °C for 14 days to allow the mycelia to completely colonize the grains. and after 15 days, the grain spawn was ready for utilisation.

Substrates preparation and spawning: Chickpea and soybean straw collected from Agronomy farms of college and was cut into small pieces (3-5 am length). The substrates were chemically sanitized by adding water containing 750 ppm formaldehyde, then left outdoors for 18 hours at temperatures ranging from 40°C to 45°C. Excess water was removed by spreading the straw on a flat, inclined surface covered with a polypropylene sheet, or by placing it on a 150-mesh iron frame. Once the substrates cooled to room temperature, they were mixed with WS in different ratios: 100: 0, 75: 25, 50: 50, 25: 75 and 0: 100%. (Agro-waste: WS). After mixing inoculated with oyster mushroom spawn at a rate of 4-6% (w/w) of the wet weight of the substrate. The inoculated substrates were mixed thoroughly and packed into sterilized polypropylene bags. The bags were tied at the top and small holes were poked for air exchange. The inoculated bags were incubated in a dark room at 25–28°C with 70–80% relative humidity for 15–20 days until complete colonization by the mycelium. Once fully colonized, the bags were transferred to a fruiting chamber with light (12-hour light/dark cycles).The fruiting room was maintained at 22–26°C with a relative humidity of 85–90%.The plastic bags were cut open to expose the substrate and regular misting was done to maintain humidity and after 7–10 days, mushroom pinheads began to form, followed by mature fruiting bodies.

Harvesting: Mushrooms were harvested when the caps were fully developed but before they began to curl upwards. The yield of oyster mushrooms was recorded by weighing the fresh mushrooms from each substrate.

Statistical analysis: The variance of the mean values was analyzed using the Duncan Multiple Range Test (DMRT) with SAS software (The SAS System for Windows, Version 8.1, 1999).

RESULTS AND DISCUSSION

Growth and development of mycelium and fruiting bodies: The shortest spawn run period was observed in WS (16.88 days), followed by the mixture of CS 25% + 75% WS (18.05 days) (Table 2). However, CS 100% took a longer time (20.58 days) for mycelial growth.The WS100% required significantly fewer days (18.98) for pinhead initiation followed by SS 25% + 75% WS (20.93 days) (Table 1). In contrast, theCS 100% took a longer time (23.48 days) to pin head initiation. The longest length of stalk was observed in the SS 100% (3.80 cm) followed by SS 75% + 25 % WS (3.60 cm). In all cases, the lowest length of stalk was in CS 100 % (2.86 cm) followed by CS 75% + 25% WS (3.05 cm).The maximum width of the stalk was observed same in the CS 100% and CS 25% + 75% WS(0.85 cm) and minimum in WS 100% (0.17

Physical Parameter

Parameter	Procedure of measurement
Days required to spawn run	This refers to the number of days needed from inoculation to the mycelium has fully colonized the substrate. The process is considered complete when the entire spawn packets turns white due to the growth of the mycelium, which serves as the indicator of successful mycelium colonization.
Days required to pin head initiation	The number of days from cutting the spawn packet to the initiation of primordia was recorded.
Average length and width of stalk (cm)s	The width and length of the stalk of each fruiting body were measured from the top to the base of the stalk.
The average diameter of the cap (cm)	The cap diameter over one gram (wt.) was recorded
Number of fruiting bodies	Only well-developed fruiting bodies were considered effective and were counted, with the total number expressed per packet. Tiny fruiting bodies were excluded from the count.
Harvest flush	It is the period when a cluster of mushrooms grows and matures together. During this stage, the mushrooms are harvested together as a group.
Harvested yield	The yield refers to the number of mushrooms produced from the substrate.
Biological efficiency	The following formula is used to calculate biological efficiency $\text{Biological Efficiency (BE)} = \frac{\text{Fresh weight of mushroom}}{\text{y weight of substrate}} \times 100$

cm) followed by CS 25% + 75% WS (0.28 cm). The highest cap diameter was inthe SS 100% substrate (10.63 cm), while the lowest was in WS100% (9.03 cm) in the first flush (Table 1). The number of fruiting bodies per packet (NFBP) varied from 16.10 to 21.13 among the substrates (Table 1,2). The maximum NFBP was counted in WS100% (21.13), followed by SS 75% + 25 % WS (18.08), while the minimum number was in CS 100% (16.10).

Harvesting time, yield and biological efficiency: The first flush harvesting time ranged from 23.78 to 28.53 days, with the shortest time in WS 100%) and the longest in CS 100% (28.53 days). Similar patterns were observed for the second and third flushes, with the minimum time taken by WS 100% and the maximum time by chickpea straw 100%. The total weight of first flush fruiting bodies per beg ranged from 323.73 to 351.57 g.In the first flush, the highest yield per beg was harvested from WS 100% (351.57 g), while the lowest was from CS 100% (323.73 g). In the second and third flushes, the highest yield per beg was observed in WS 100% (302.68 and 247.85 g) but the lowest yield per beg was observed in SS 100% (257.60 g) (Table 1) in the second flush and in third flush CS 100% produce minimum yield per beg (174.98 g) (Table 2).In all cases, maximum average total yield and biological efficiency were obtained from WS 100% (902.10 g and 90.21%) followed by SS 75% + 25 % WS (865.08 g and 86.51%) and the lowest was from chickpea straw100% (764.30 g and 76.43%).

The observed variations in mushroom growth and yield across different substrate ratios can be linked to the differences in the chemical composition and physical properties of the substrates. The higher mycelial growth and faster spawn running observed in WS 100% may be due to its high cellulose and lignin content, which provides an ideal structure and nutrient base for oyster mushroom growth. Previous studies have confirmed that WS supports robust mycelial colonization and consistent yields in oyster mushrooms (Royse et al., 2017). It provides a balanced carbon-to-nitrogen ratio and an optimal lignocellulosic structure, which facilitates the breakdown of complex compounds by mushroom enzymes (Ritota and Manzi 2019). The favorable nutrient composition and physical structure in WS 100% may have supported rapid colonization (Wan-Mahari et al., 2020). Similarly, the faster pinhead initiation with WS and SS mixtures is supported by findings from other studies that confirm WS's superiority in inducing quicker fruiting (Fernandes et al., 2015). The substrates like CS took longer to achieve full colonization and pinhead formation, which could be due to differences in their lignocellulosic

Table 1. Effect of soybean straw on yield contributing characters of *P. membranaceus*

Sr. No.	Treatments	Spawn run (days)	Pinhead initiation (days)	Stipe length (cm)	Stipe width (cm)	Cap diameter (cm)	No. of fruiting bodies	Flush time (days)			Flush yield (g)			Average total yield (g)	Biological efficiency (%)
								1st	2nd	3rd	1st	2nd	3rd		
T_1	SS 100 %	20.10^{a}	22.70^{a}	3.80^{a}	0.75^{d}	10.63^{a}	16.40^{d}	27.05^{a}	42.88^{a}	57.40^{a}	334.70^{e}	257.60^{e}	193.13^{e}	785.43^{e}	78.54^{e}
T_2	SS 75% + 25 % WS	19.70^{ab}	22.30^{ab}	3.60^{ab}	0.77^{c}	10.33^{a}	18.08^{b}	26.58^{ab}	41.88^{b}	56.58^{b}	339.33^{b}	288.08^{b}	237.68^{b}	865.08^{b}	86.51^{b}
T_3	SS 50% + 50 % WS	19.15^{b}	21.75^{b}	3.48^{abc}	0.82^{b}	9.85^{b}	17.63^{bc}	26.03^{b}	42.18^{b}	56.03^{b}	337.63^{c}	286.98^{c}	220.85^{c}	845.45^{c}	84.55^{c}
T_4	SS 25% + 75 % WS	18.33^{c}	20.93^{c}	3.38^{bc}	0.85^{a}	9.35^{c}	17.03^{cd}	24.93^{c}	40.18^{c}	55.15^{c}	335.68^{d}	269.93^{d}	206.75^{d}	812.35^{d}	81.24^{d}
T_5	WS 100 %	16.88^{d}	18.98^{d}	3.18^{c}	0.17^{e}	9.03^{c}	21.13^{a}	23.78^{d}	37.95^{d}	53.40^{d}	351.57^{a}	302.68^{a}	247.85^{a}	902.10^{a}	90.21^{a}

SS, Soybean straw; WS, Wheat straw

Table 2. Effect of chickpea straw on yield contributing characters of *P. membranaceus*

Sr. No.	Treatments	Spawn run (days)	Pinhead initiation (days)	Stipe length (cm)	Stipe width (cm)	Cap diameter (cm)	No. of fruiting bodies	Flush time (days)			Flush yield (g)			Average total yield (g)	Biological efficiency (%)
								1st	2nd	3rd	1st	2nd	3rd		
T_1	CS 100%	20.58^{a}	23.48^{a}	2.86^{c}	0.85^{a}	10.40^{a}	16.10^{c}	28.53^{a}	44.18^{a}	58.03^{a}	323.73^{e}	262.93^{d}	174.98^{e}	764.30^{e}	76.43^{e}
T_2	CS 75% + 25 % WS	20.20^{a}	23.13^{a}	3.05^{b}	0.65^{b}	9.70^{b}	17.83^{b}	27.05^{b}	42.90^{b}	57.18^{a}	328.33^{b}	286.98^{b}	229.75^{b}	845.05^{b}	84.51^{b}
T_3	CS 50 % + 50 % WS	19.20^{b}	22.10^{b}	3.13^{ab}	0.45^{c}	9.55^{b}	16.35^{c}	25.95^{c}	41.40^{c}	55.90^{b}	326.30^{c}	274.63^{c}	220.90^{c}	821.83^{c}	82.18^{c}
T_4	CS 25% + 75 % WS	18.05^{c}	20.95^{c}	3.18^{a}	0.28^{d}	9.45^{bc}	16.68^{c}	24.80^{d}	40.35^{d}	54.70^{c}	325.70^{d}	263.63^{d}	206.20^{d}	795.53^{d}	79.29^{d}
T_5	WS 100%	16.88^{d}	18.98^{d}	3.18^{a}	0.17^{e}	9.03^{c}	21.13^{a}	23.78^{e}	37.95^{e}	53.40^{d}	351.57^{a}	302.68^{a}	247.85^{a}	902.10^{a}	90.21^{a}

CS, Chickpea straw; WS, Wheat straw

structure. CS is known to have a denser composition, which may slow down the breakdown by fungal enzymes, extending the time for mycelial spread and fruiting (Fufa et al., 2021).

The SS produced the longest stalks and largest cap diameters also aligns with literature suggesting that soybean residues provide more nitrogen, contributing to the larger fruiting bodies (Ramos et al., 2011). The lower stalk lengths in chickpea substrates may result from their lower nitrogen and nutrient availability, leading to smaller fruit bodies (Fufa et al., 2021). The highest number of fruiting bodies per packet (NFBP) in WS can be explained by its balanced nutrient profile, which supports higher biological efficiency and yield (Dubey et al., 2019). Conversely, the lower NFBP in CS points to substrate limitations in supporting extensive fruiting, a pattern also observed in other substrate experiments (Maheshwari et al., 2021).

The substrates composed entirely of WS 100% resulted in higher yields, indicating that WS plays a crucial role in supporting mushroom growth. This may be due to the structural characteristics of WS, which provide better aeration and water retention properties, as well as its favorable carbon-to-nitrogen ratio (Zhang et al., 2014). Substrates with imbalanced ratios may impede efficient mycelial colonization and fruiting body formation., SS 75% + 25 % WS mixtures produced particularly favorable results after the WS 100%, Aske et al. (2020) suggesting that a balanced substrate mixture can optimize mushroom growth. Singh et al. (2019) also observed that combining multiple agro-wastes could enhance substrate utilization efficiency. This might be attributed to a synergistic effect that improves enzyme activity, resulting in better breakdown of cellulose and hemicellulose.

CONCLUSION

This study confirms that substrate choice significantly impacts the growth and yield of *P. membranaceus*. WS, either alone or in combination with soybean straw, proved to be the most effective substrate, offering faster mycelial colonization, early pinhead initiation and higher fruit body yields. In contrast, chickpea straw, though high in protein, showed slower mycelial growth and lower yields, indicating it is less suitable for oyster mushroom cultivation without further optimization. These findings suggest that WS remains the most viable substrate for commercial cultivation, while SS can enhance specific growth parameters such as cap size and stalk length. Further research is recommended to explore alternative substrate combinations to improve sustainability and yield efficiency.

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Seasonal Variations of Heavy Metal in Seawater and Sediment from Uppanar Estuary, Cuddalore Coast, India

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Abstract: The current study was carried out to estimate of heavy metal concentration in seawater and sediment samples from industrial area of Uppanar estuary, Cuddalore. Seawater and sediment samples were collected in two different stations during four seasons (post monsoon, summer, pre monsoon and monsoon). The heavy metal concentration was higher in sediment samples were compared with water samples in monsoon season compare with other seasons. The order of heavy metal concentration in seawater and sediment samples was Fe > Cu > Zn > Cd > Cr > Ni.

Keywords: Uppanar estuary, Seawater, Sediment, Cuddalore coast, Heavy metal

The contamination of aquatic and terrestrial ecosystems with heavy metals is a major environmental problem. Some of these metals are potentially toxic or carcinogenic at sufficient concentrations and can cause serious human health hazards if they enter the food chain. A lot of input eventually accumulates in the estuarine zone and continental shelf, which are important sinks for suspended matter and associated land-derived contaminants. (Masindi 2018, Walker et al., 2012). The pollution of aquatic and terrestrial ecosystems with heavy metals is a major environmental trouble. Some of these metals are potentially toxic or carcinogenic at enough concentrations and can cause serious human health hazards if they enter the food chain. Heavy metals in the sediment are essential to assess the extent of metal pollution. The distribution of heavy metals in solution has widely been recognized as a major factor in the geochemical behavior, transport and biological effects of these elements in natural waters (Karthikeyan et al., 2004, Ananthan et al., 2006, Karthikeyan et al., 2007).

The majority of the heavy metals are essential for growth of organisms, but are only required in low concentrations (Akpoveta et al., 2010). The increasing concentration of heavy metals leads to bioaccumulation of metals in fauna and flora if the rate of uptake of heavy metals by the organisms is more than the excretory phase. Heavy metals are not biodegradable, so they accumulate in primary organs in the body and over time begin to fester, leading to various symptoms of diseases (Siyanbola et al., 2011). Thus, untreated or incompletely treated industries effluent can be harmful to both aquatic and terrestrial life by unfavorably affecting on the natural ecosystem and long term health effects. Such accumulation takes place by biological and geochemical mechanisms (Karbassi and Amirnezhad 2004). Evaluation of heavy metal concentration in the coastal waters can be made by using indicator organisms which accumulate pollutants proportionally to their environmental condition (Thangaradjou et al., 2010).

Heavy metals have been renowned to interact with nuclear proteins together with DNA which cause specific damage. Two types of damages may be caused by direct and indirect damage. In direct damage, conformational changes occur to the biomolecules, due to the metal. The heavy metal causes indirect damage, which is a result of the production of immediate oxygen and nitrogen species which comprise of the hydroxyl and superoxide radicals, hydrogen peroxide, nitric oxide and other endogenous oxidants. Heavy metals also activate signaling pathways (Valko et al., 2005). These are the basic steps used in sewage treatments depending on what the sewage contains and where it is being process. Several controls have been set up due to the problems caused by sewage exclusion into the rivers and seas without being treated. Severe regulations have been placed and better technology has been developed to decrease the amounts of pollutants that are unnerved in the waters (Walker et al., 2012).

Chromium is a cancerous and toxic element and is used in many industries that pose a threat to regional climates. In assessment to natural chromium emissions from the environment, ferrochrome industry emissions are at the highest level (Coetzee et al., 2020). Nickel is a naturally abundant element and has extensive industrial uses. It is emitted from both natural and anthropogenic sources into the atmosphere (Li et al., 2016a). It has many unpleasant effects on humans, and causes allergies, nasal and lung cancer, and kidney and cardiovascular diseases owing to the inhalation of contaminated air (Genchi et al., 2020, Lu et al., 2005). The present study focus on the level of dissolved trace metals in coastal water off Cuddalore.

MATERIAL AND METHODS

Study area: The Uppanar estuary is situated in Cuddalore (Lat.11/ 43'N, Long. 79/ 46' E). (Fig. 1) is at an average height of about 1 m above sea level. It is brief through the Industrial coastal town of Cuddalore on the southeast coast of India. The major 55 industries along the Uppanar estuary contain chemicals; beverage industrialized, tanneries, oil, soap, paint production, etc. Two different sampling sites have been selected for this study.

Sampling site I- Uppanar estuary (SIPCOT): The Uppanar estuary runs following 1 km away from the mouth of the estuary SIPCOT (State Industrial Promotion Corporation of Tamil Nadu) industrial complex is located in Cuddalore which consists of many chemicals, plastics, soap, pharmaceutical industries, etc. This open type of estuary receives treated and untreated industrial waste containing toxic wastage. In addition to the estuary to receives also domestic and municipal waste in Cuddalore old town.

Sampling site II- Sea mouth region (Fishing harbor): The station 2 adjacent to the mouth of the estuary located on near the Cuddalore old town all the discharged industrial, municipal, agriculture and domestic waste are mixed and diluting the sea mouth region.

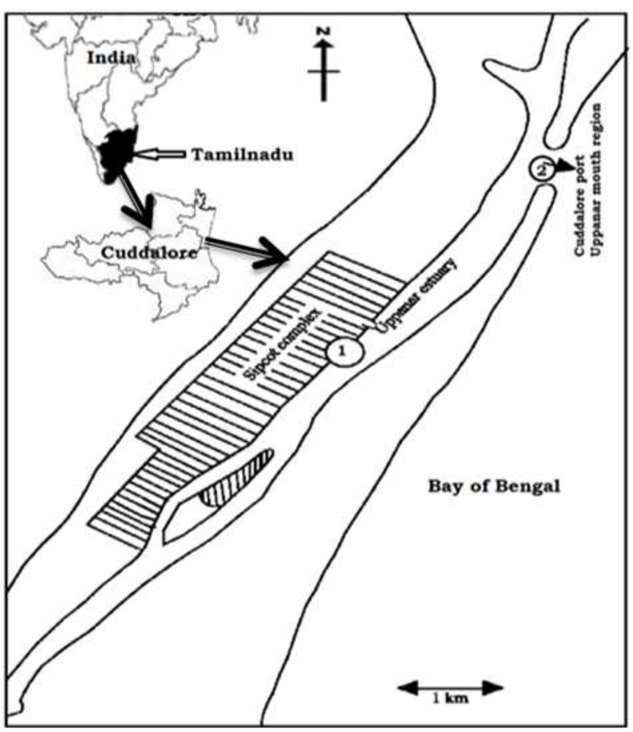

Fig. 1. Sampling sites on the Cuddalore coast

Sample collection and maintenance: The sample collection was made during forenoon from January to December 2012 for four seasons viz. post-monsoon (January-March), summer (April-June), pre-monsoon (July-September) and monsoon (October-December) from two sites in using wide mouth polyethylene bottle regularly. The collected seawater sample safely transported to the laboratory condition in ice box and preserved refrigerator for 4°C till their use. The sediment samples (1 meter depth) were scraped in pre cleaned zip lock polyethylene cover after collection the sediment samples were brought to the laboratory condition and air dried for 5 days awaiting reaches a stable weight.

Heavy metal analysis in seawater samples: The collected seawater samples taken 1 litter were filtered through Whatman filter paper No. 42 and adjusted to pH 2 with HNO_3 taken in a separating funnel. 10 ml (3% w/v) of a freshly prepared solution of ammonium-pyrolidine dithiocarbamate (APDC) added into the funnel, and the mixture was shaken by a mechanical shaker for10 min. Further, 25 ml of iso-butyl methyl ketone (MIBK) added to this mixture and shaken for 15 min. The phases were allowed to separate. The top organic phase was collected. The bottom aqueous phase again shaken with 25 ml of MIBK, and the organic phase obtained then pooled with an earlier one. The pooled organic phase mixed with 2 ml of 50% HNO3 and shaken vigorously for 10 min to separate the bottom acid layer and make up 25 ml of metal free distilled water (Jonathan et al., 2008).

Heavy metal analysis in sediment samples: Heavy metal extraction in sediment samples with slightly modified. The dried sediment samples were ground well in a mortar and pestle it weighed in 1.00 g of dried sediment sample was taken with a mixture of 1,5 and 2 ml of H_2SO_4, HNO_3 and $HClO_4$. The mixture was boiled on a hot plate, evaporated to near dryness. At the end of the digestion, the samples were making up 25 ml of metal free double distilled water and filtered through Whatman No.42 filter paper. The filtered sample was aspirated into the Atomic Absorption Spectrophotometer (AAS) and the reading was taken and the quantified metals are expressed in parts per million (ppm).

Statistical analysis: All the data were analyzed statistically apply using software PAST (version 3) and SPSS (version 16) for the studied parameters.

RESULTS and DISCUSSION

Seasonal variations of heavy metals in seawater samples: Iron concentration of seawater sample value varied from 2.53 ppm to 3.21 ppm (St. 1) and 2.21 ppm to 2.89 ppm (St. 2). The highest was during the monsoon season and lowest value during the summer season. The seasonal correlation matrix indicated for Fe with Cu and cadmium in the sediment is highly significant in St.I. Copper in water values ranged from 1.86 to 2.19 ppm (St. 1) and 1.72 to 2.01ppm (St. 2). The highest value observed during post-monsoon and monsoon season and the lowest value was during the summer season.

Zinc in water content varied from 1.65 to 2.10 ppm (St. 1) and 1.53 to 2.01 ppm. The highest was observed in post-monsoon and monsoon season and the lowest during the summer season. Cadmium in sea water values ranged from 1.38 to 1.53 ppm (St. 1) and 1.32 to 1.46 ppm (St. 2). The maximum was observed during the monsoon period and the smallest value was observed during the summer season. Chromium in water concentration varied from 1.42 to 1.65 ppm (St.1) and 1.36 to 1.56 ppm (St.2). The highest was recorded during monsoon season and the lowest was observed during the summer and pre-monsoon seasons. Nickel in sea water values ranged from 1.21 to 1.31 ppm (St.1) and 1.18 to 1.28 ppm (St.2). The high nickel value was observed during the monsoon period and the lowest value was observed during the summer season in both study sites (Table 1).

Seasonal variations of heavy metals in sediment samples: Higher metal values recorded in sediment samples were compared with water samples. The iron present in the sediment sample varied from 2.50 to 4.80 ppm (St.1) and 2.20 to 4.20 ppm (St.2). The highest was observed during the monsoon season and lowest during the summer season. The amount of copper present in the sediment sample ranged from 1.86 to 2.21 ppm (St.1) and 1.69 to 2.05 ppm (St.2). The highest values were observed during the monsoon season and lowest value was observed during the summer season. Zinc concentration of sediment samples varied from 1.68 to 2.25 ppm (St.1) and 1.00 to 1.25 ppm (St.2). The highest were observed during the monsoon season and lowest during the summer and pre-monsoon seasons. The amount of cadmium present in the sediment sample ranged from 1.31 to 1.51 ppm (St.1) and 1.26 to 1.41 ppm (St.2). The highest cadmium was observed during the monsoon season and lowest during the summer season. Chromium concentration in sediments varied from 1.48 to 1.67 ppm (St.1) and 1.42 to 1.56 ppm (St.2). The high chromium concentration was observed during the monsoon season and lowest during the summer season. Nickel concentration in the sediment sample values ranged from 1.33 to 1.63 ppm (St.1) and 1.27 to 1.52 ppm (St.2). The highest value was observed during the monsoon season and lowest during the pre-monsoon season (Table 2).

The concentrations of heavy metals in water and sediment varied depending on their incidence and seasonal difference. Generally, the natural source of heavy metal pollution in coastal waters through land derived run-off, chemical and mechanical weathering of rocks. The components were also washed from the atmosphere through rainfall, windblown dust, forest fire and volcanic particles (Sankar et al., 2010). The highest concentration of heavy metals was observed in sediment sample as compared with water samples. The high concentration of heavy metals in sediment samples depends on the migration of the water column downwards in sediment. Highest metal contents were Fe, Cu and Zn in monsoon and post monsoon season. Maximum iron concentration observed in monsoon closely followed by a post monsoon season. The season of abundance of metal is due to run off water into the sampling sites. This was substantiated in sediment sample which also exhibited similar trends of results. The correlation matrix of water samples during all seasons was significantly different. The correlation matrix indicated that iron with copper and cadmium in the sediment were significant ($r= 0.96$; $r= 0.98$) in St.I (Table 3).

The copper (Cu) is a normal element which is widely

Table 1. Heavy metals in water sample (Mean ± Standard deviation)

Heavy metals	Stations	Fe	Cu	Zn	Cd	Cr	Ni
Post monsoon	S1	3.10±1.0	2.12±1.07	2.09±0.99	1.49±0.98	1.57±0.95	1.24±0.94
	S2	2.65±1.0	1.98±0.95	2.00±0.90	1.39±0.89	1.51±0.87	1.19±0.87
Summer	S1	2.53±1.0	1.86±0.93	1.65±0.89	1.38±0.87	1.42±0.84	1.21±0.83
	S2	2.21±1.0	1.72±0.86	1.53±0.83	1.32±0.81	1.36±0.79	1.18±0.78
Pre monsoon	S1	2.60±1.0	1.96±096	1.76±0.91	1.41±0.89	1.51±0.87	1.25±0.86
	S2	2.32±1.0	1.82±0.88	1.65±0.84	1.35±0.83	1.43±0.81	1.21±0.80
Monsoon	S1	3.21±1.0	2.19±1.10	2.10±1.02	1.53±1.0	1.65±0.97	1.31±0.96
	S2	2.89±1.0	2.01±1.02	2.01±0.95	1.46±0.94	1.56±0.91	1.28±0.90

spread in soils and rock sand in rivers and the sea. High copper value was observed during the monsoon season and lowest value was observed during the summer season in both St.1 and St.2. The copper concentration at the outfall increased twofold compared to the outlet due to the anthropogenic activity, agricultural run-off; industrial and domestic waste water into the river. Copper is released into water as a result of ordinary weathering of soil and discharges from industries and sewage treatment plants (Hutchinson 2002). Copper concentration in downstream was several than higher than the raw effluents and may be attributed to domestic sewage and runoff from extensive farmed areas (Wu et al., 2008). Copper has a positive correlation coefficient with iron (Table 3).

Zinc concentration has always had a tendency to couple with organic carbon. Decomposition of the organic substance release heavy metals reverse to sediments and accumulated, and this process might be responsible for the strong association of zinc and copper with organic carbon (Bardarudeen et al., 1996). The highest organic carbon observed during the post-monsoon and monsoon seasons coincide with the elevated level of zinc and copper in sediments. Besides, the release of heavy metals through the influx of land-derived contaminant increased the level of zinc and copper despite they are meagre in amount.

Generally, zinc and copper are good indicators of anthropogenic inputs.

High cadmium ranges were observed in monsoon and lowest in summer season, which could be attributed to the particulate fractions derived from the river run-off caused by

Table 2. Heavy metals in sediment sample (Mean ± Standard deviation)

Heavy metals	Stations	Fe	Cu	Zn	Cd	Cr	Ni
Post monsoon	S1	4.3±1.0	2.18±1.0	2.25±1.0	1.42±1.0	1.58±1.0	1.52±1.0
	S2	3.6±1.0	2.0±1.0	2.10±1.0	1.38±1.0	1.49±1.0	1.43±1.05
Summer	S1	2.5±1.0	1.86±1.0	1.68±1.0	1.31±1.0	1.48±1.0	1.38±0.81
	S2	2.7±0.57	1.69±1.0	1.61±1.0	1.27±1.0	1.41±1.0	1.35±1.06
Pre monsoon	S1	2.9±1.0	1.87±1.0	1.79±1.0	1.39±1.0	1.51±1.0	1.33±0.88
	S2	2.2±1.0	1.78±1.0	1.63±1.0	1.31±1.0	1.42±1.0	1.27±1.0
Monsoon	S1	4.8±1.0	2.21±1.0	2.13±0.95	1.51±1.0	1.68±1.0	1.61±1.36
	S2	4.2±1.0	2.25±1.0	2.10±1.0	1.42±1.0	1.57±1.0	1.52±1.0

Table 3. Correlation-coefficients matrix (*r*) of heavy metals in seawater and sediment samples during different seasons at station I

		Water sample						Sediment sample					
	Heavy metals	Fe	Cu	Zn	Cd	Cr	Ni	Fe	Cu	Zn	Cd	Cr	Ni
Water sample													
	Fe	1.00											
	Cu	0.893	1.00										
	Zn	0.690	0.719	1.00									
	Cd	0.986*	0.938	0.784	1.00								
	Cr	0.770	0.965*	0.784	0.857	1.00							
	Ni	0.847	0.625	0.823	0.845	0.545	1.00						
Sediment sample													
	Fe	0.975*	0.967*	0.679	0.982*	0.873	0.735	1.00					
	Cu	0.887	0.999**	0.687	0.929	0.960*	0.598	0.966*	1.00				
	Zn	0.706	0.756	0.998**	0.802	0.821	0.806	0.707	0.726	1.00			
	Cd	0.994**	0.935	0.730	0.997**	0.835	0.825	0.989*	0.928	0.750	1.00		
	Cr	0.687	0.919	0.808	0.793	0.990*	0.500	0.799	0.910	0.844	0.762	1.00	
	Ni	0.860	0.574	0.682	0.821	0.441	0.975*	0.728	0.552	0.664	0.818	0.369	1.00

**correlation is significant at the 0.05 and 0.01 level

Table 4. Correlation-coefficients matrix (*r*) of heavy metals in seawater and sediment during different seasons at station 2

		Water sample						Sediment sample					
	Heavy metals	Fe	Cu	Zn	Cd	Cr	Ni	Fe	Cu	Zn	Cd	Cr	Ni
Water sample													
	Fe	1.00											
	Cu	0.899	1.00										
	Zn	0.770	0.737	1.00									
	Cd	0.995**	0.932	0.739	1.00								
	Cr	0.781	0.945	0.839	0.808	1.00							
	Ni	0.894	0.652	0.832	0.844	0.588	1.00						
Sediment sample													
	Fe	0.971*	0.968*	0.706	0.990**	0.849	0.765	1.00					
	Cu	0.938	0.995**	0.738	0.964*	0.915	0.710	0.988*	1.00				
	Zn	0.774	0.764	0.998**	0.749	0.868	0.812	0.724	0.761	1.00			
	Cd	0.981*	0.878	0.631	0.986*	0.704	0.825	0.970*	0.922	0.637	1.00		
	Cr	0.715	0.904	0.839	0.742	0.994**	0.535	0.787	0.866	0.869	0.624	1.00	
	Ni	0.866	0.564	0.685	0.813	0.436	0.974*	0.725	0.639	0.659	0.833	0.365	1.00

**correlation is significant at the 0.05 and 0.01 level

monsoonal flow, and occurrence of relatively higher percentage of particulate matter could be due to the heavy rainfall and river run-off received from industrial and land-derived contaminants along with domestic and municipal waste which includes heavy metal-containing pesticides (Karthikeyan et al., 2004, Ananthan et al., 2005,2007). Cadmium has a positive correlation with iron in St.1 and St.2 water samples (Table. 3) and also in sediment samples . Cd is a component of pesticides and fertilizers. The volatilization of Cd from fertilized agricultural lands introduces significant amounts of Cd into the atmosphere, which, through runoff, gets into the aquatic ecosystem (Annema et al., 2016).

The highest chromium and nickel were observed during monsoon season and lowest value was recorded in summer season at both stations. The high chromium during monsoon could be attributed to the Palar river discharge as it carries tannery and distillery effluents from the upstream (Velsamy et al., 2013). Lowest chromium concentration recorded during summer season might be due to the smaller amount or lack of rainfall, which eventually reduces the quantity of river flow.

The estuaries are highly active systems from both a physical and chemical point of view. Sharp gradients in parameters such as temperature, salinity, pH and dissolved oxygen induce considerable biochemical activity and the behaviour of trace elements in the estuarine ecosystems (Wang et al., 2007, Satheeskumar 2011). Dissolved heavy metals such as copper and zinc present in a maximum quantity at relatively high salinity and decreased seawater Cadmium.

CONCLUSION

The heavy metal concentration in the seawater and sediments from the Uppanar estuaries has recognized that Cu, Zn, Cd, Cr and Ni are present by the change degrees of absorption. It is supposed that the level of absorption that the defect of above metals will continue to increase in Uppanar estuary. In the current study all the metals are originate in higher concentration in the upstream side of the estuary, anywhere additional industries are united along the estuary areas. In this area majority of the industrial wastage, agricultural wastes which are treated or unprocessed waste is released into the estuary, the water flow in this area is stagnant or flow of water will be smaller quantity, which lead to contamination and destroy the ecosystem. Contamination of irrigate by the chemical substance has critically affected the human healthiness because most of these heavy metals cause serious illness.

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Rock-Water Interactions and its Hydrogeochemical processes of Groundwater Resources: A Case Study for Mandya District of Karnataka State, India

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***Abstract*:** Rock-Water interactions and hydrogeochemical processes are critical for understanding groundwater chemistry and the environmental impacts of various geological formations. These interactions involve several chemical processes that significantly influence the composition and groundwater quality. The present study aims to investigate the rock-water interactions and hydrogeochemical processes through the water samples collected from Mandya district. The study involved Piper's diagram, Gibb's diagram and Scatter plots of various parameters in revealing chemical processes that influence hydro geochemistry. The concentrations of these ions varied spatially and temporally in different taluks of Mandya district, depending on the lithological formations of those locations. Ions were dominated by Na>Ca>Mg>K = HCO_3>Cl>SO_4>CO_3. Ca-Mg-Cl, Ca-Na-HCO_3, and Ca-HCO_3 types were the dominant hydro chemical facies noticed in the study area. Hydrogeochemical processes provide valuable insights into the complex systems of rock-water interaction and to aid in effective groundwater management and pollution mitigations.

***Keywords*:** Rock-Water Interactions, Hydrogeochemical processes, Mandya

Groundwater contributes to about eighty percent of the drinking water requirements in the rural areas, fifty percent of the urban water requirements and more than fifty percent of the irrigation requirements of the nation (CGWB 2012). Rock-water interaction is the complex chemical and physical processes that occur when groundwater interacts with geological materials. Rainwater enters the ground's surface, penetrates it, and runs through the soil and rock zones (Yousif and Aassar 2018). Several chemical reactions occur during groundwater migration, depending on the chemical makeup of the water, the rock in its flow path, and the amount of time spent in residence (Yousif and Aassar 2018). This interaction is crucial in hydrogeology, as it alters the chemical composition of groundwater, which is influenced by the minerals present in the rocks (Yousif and Aassar 2018). Minerals within rocks can dissolve in water, releasing ions into the groundwater and even precipitate out of solution, forming new minerals (Ye et al., 2024). This process involves the exchange of ions between the water and the mineral surfaces, affecting the concentration of various chemical species in the groundwater (Rajmohan and Elango 2003). Oxidation and reduction reactions can alter the mobility of certain elements, particularly metals, influencing groundwater quality and contaminant transport (Basavarajappa et al., 2015c).

The mineral composition of hard to soft rocks (igneous, sedimentary, or metamorphic) affects how easily they interact with water. Limestone (sedimentary) is more susceptible to dissolution than granite (igneous). The initial chemical composition of the groundwater plays a critical role in determining the extent and nature of rock-water interactions (Elango and Kannan 2007). Groundwater can be classified into various types based on its chemical constituents, such as Ca-HCO_3, Na-Cl, and Ca-SO_4, which reflect the dominant rock-water interactions occurring in each area. Factors like temperature, pressure, and the presence of organic materials can influence the rates and types of chemical reactions that occur during rock-water interactions (Rasool and Ahmad 2023). Rock-Water interaction and hydrogeochemical processes are key to determining the quality of groundwater resources, which are vital for drinking water, agriculture and industrial suitability. Knowledge of these processes helps in assessing the impacts of human activities, such as mining and agriculture, on groundwater systems and in developing strategies for pollution mitigations (Li et al., 2021). Insights into rock-water interactions are crucial for the exploration and management of natural resources, including minerals and hydrocarbons.

MATERIAL AND METHODS

Site description: Mandya district is in the south-eastern part of Karnataka State with geographical area of 4,850 km^2 and situated between 12^{0}13' to 13^{0}04' latitude and 76^{0}19' to 77^{0}20' longitude (Fig. 1) (Sagar et al., 2024). It comprises seven taluks namely Krishnarajpet, Maddur, Malavalli, Mandya, Nagamangala, Pandavapura, and Srirangapatna with elevations ranged from 600 to 1045 mts above mean sea level (Sagar et al., 2024). The district forms the part of the southern maiden area, which comprises of broad undulating plateau and slopes towards the southeast. It enjoys tropical to sub-tropical climate with temperatures ranging from 16^0 to 37^0C with an average annual rainfall of 750 mm. The hottest month is April, and the temperature drops significantly as the southwest monsoon arrives in June and reaches coldest during December. The district falls under the rain shadow zone of Western Ghats and receives most of its rainfall during monsoon seasons (Thimme Gowda et al., 2015). Most of the district is covered by red sandy soil, followed by red clay soil, medium black soil and lateritic soil.

Agriculture and irrigation: Cauvery is the major river flowing towards east direction along with its tributaries namely, Hemavathi, Shimsha, Lokapavani and Veeravaishnavi (Begum and Harikrishnarai, 2008). Apart from these rivers, the district is endowed with number of streams, which along with the rivers form sub dendritic drainage patterns. The Krishnaraja Sagara reservoir was built for the Cauvery River, which is the primary source of water for irrigation in much of Maddur, Mandya, Srirangapattana, Pandavapura, and Mallavalli taluks (CGWB 2012). It is predominantly an agricultural district, benefitting from irrigation from the River Cauvery for the major crops of paddy, ragi, jowar, maize, pulses, oil seeds, sugarcane, cotton, fruits and various vegetables (CGWB 2012). K.R pete, Nagamangala and some sections of Mandya and Mallavalli taluks are dry areas and rely on groundwater for irrigation. Mandya is one of the most fertile districts of the state, and various surface and groundwater plays an important role in irrigating (CGWB 2012).

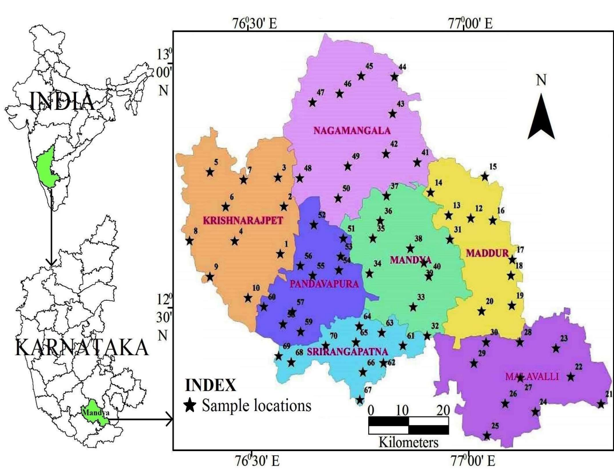

Fig. 1. Groundwater samples location map of Mandya district

Geological settings: The study area falls under Western Dharwar Craton and Peninsular Gneiss and comprises 3.4-3.0 Ga ancient 'Supracrustals' (Sargur Group) and tonalite-trondhjemite-granodiorite (TTG) basement overlain unconformably by 2.9-2.6 Ga greenstone belts (Radhakrishna and Vaidyanathan 1997). Gneiss, granite, pegmatite, and ultramafic rock & dykes are the major rock types observed during limited field visits (Fig. 2a) (Sarbajna et al., 2018). Gneiss, granite, amphibolite schist, and excess mica mineral are identified near Melukote area (Babitha Rani et al., 2015). The Peninsular Gneisses cover almost 80% of the areal extent of Mandya district (Suresha 2016). Small patches of porphyritic granite of Closepet age are also exposed (Sukanta Dey et al., 2003). From the groundwater point of view, these rocks are classified as crystalline formations (Baiocchi et al., 2016). The fracture/fissure system formed along with joints and faults traversing the rocks facilitates groundwater circulation and hols a moderate amount of water (Basavarajappa et al., 2015b). Groundwater quality is determined by the mineralogical composition of the rocks. Conglomerate, amphibolite, pelitic schist, quartzite, iron formation, small carbonate bodies, and volcanic rocks from the Nagamangala schist belt were also encountered. In the metasediments, bedding planes and the fractures facilitate water movement and accumulation (CGWB 2012). The schistose rocks are poor aquifers and yield water of poorer quality in very less quantity. Groundwater generally occurs in the water table conditions in the weathered and decomposed mantle and under semi-confined conditions in the deeper fractures (CGWB, 2012). There are few sporadic outcrops of rocks where Cauvery River breaks through the hills ranges with waterfall and few fertile shallow valleys.

Data collection & analysis: The methodology for water samples collection was designed to ensure that the samples accurately reflect the groundwater chemistry and the interactions occurring with surrounding rocks. 70 groundwater samples were collected at random on geological features, proximity to potential contamination sources, areas with known mineral deposits and hydrological characteristics (Sagar et al., 2024). Groundwater was collected in sterile, clean polyethylene sampling bottles to determine the concentration of water quality parameters using WHO and BIS (Basavarajappa et al., 2015a). The same day, the bottles were washed with distilled water and rinsed with the same water samples and sent to the laboratory (Sagar et al., 2024). The groundwater was filled up to about 80% of bottle capacity to allow for expansion and prevented overflow. Garmin GPS etrex-10 portable equipment was utilized in recording each sample locations precisely within the district during the Pre-monsoon period (April) of the year 2023 (Fig. 1) (Manjunatha and Basavarajappa 2015). The bottles were stored in a cooler with ice packs during transportation to the laboratory to maintain temperature and prevent changes in water chemistry, since these were collected during summer season of 2023 (Sagar et al., 2024).

Samples were treated with nitric acid for cation analysis and kept at 4^0C for anion analysis. The present analysed parameters include electrical conductivity (EC), potential of hydrogen (pH), and cation groups like calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), potassium (K^+), and anions groups like bicarbonate (HCO_3), carbonate (CO_3^{2-}), sulphate (SO_4^{2-}), and chloride (Cl^-) (Sagar et al, 2024). EC, pH, and TDS were estimated in the field by using a Hanna field meter; Ca^{2+}, Mg^{2+}, and Cl^- were measured using a volumetric titration method; Na^+ and K^+ were determined using a Flame Photometer; F- was estimated using a visual interpretation technique; and SO_4^{2-} was recorded using a turbidity method in accordance with BIS Standard (Ramesh and Elango 2012). Scatter plots for geochemical modelling was performed using Sigma plot software in understanding the processes of rock-water interactions and predicting groundwater behaviour (André et al., 2005).

RESULTS AND DISCUSSION

Hydrochemistry: EC concentration ranged from 291 to 2472 µS/cm, while the pH ranged from 6.7 to 8. Ion's dominance in the district followed the order of Na> Ca> Mg>K = HCO_3>Cl>SO_4>CO_3 (Table 1). The concentration of major anions and cations were observed in the collected water samples through Piper's diagram (Fig. 2b) (Sircar et al., 2022). Another most used graphical technique that is crucial for the reduction of chemical data is the Piper trilinear diagram (1994). It consists of a diamond-shaped quadrilateral situated between two neighbouring equilateral triangles. Ca-Mg-Cl, Ca-Na-HCO_3, and Ca-HCO_3 were the three hydrogeochemical facies types of Groundwater noticed in Mandya district. Ca-HCO_3 (calcium-carbonate) is the superior form of water among the collected samples, indicating that the above water type is the result of groundwater replenishment and agricultural water return flow. The chemical composition of groundwater is determined by the interaction of rock and water (Jiang, et al., 2023).

Major Ion Chemistry and Chemical Processes

Calcium and magnesium: Calcium is the second most dominant cation noticed among the collected water samples that ranged from 14.4mg/l to 144mg/l. Calcium, sodium, and magnesium are associated with minerals such as

Table 1. Minimum and maximum values of major ion concentration

Unit	Milligram per Litre (mg/l)								EC(µS/cm)
Parameters	Ca	Mg	Na	K	CO_3	HCO_3	Cl	SO_4	
Minimum	14.4	6.4	21.95	0.28	0.1	94.9	13	2.98	291
Maximum	144	111.6	276.65	78.95	5.49	900.3	254	251	2472

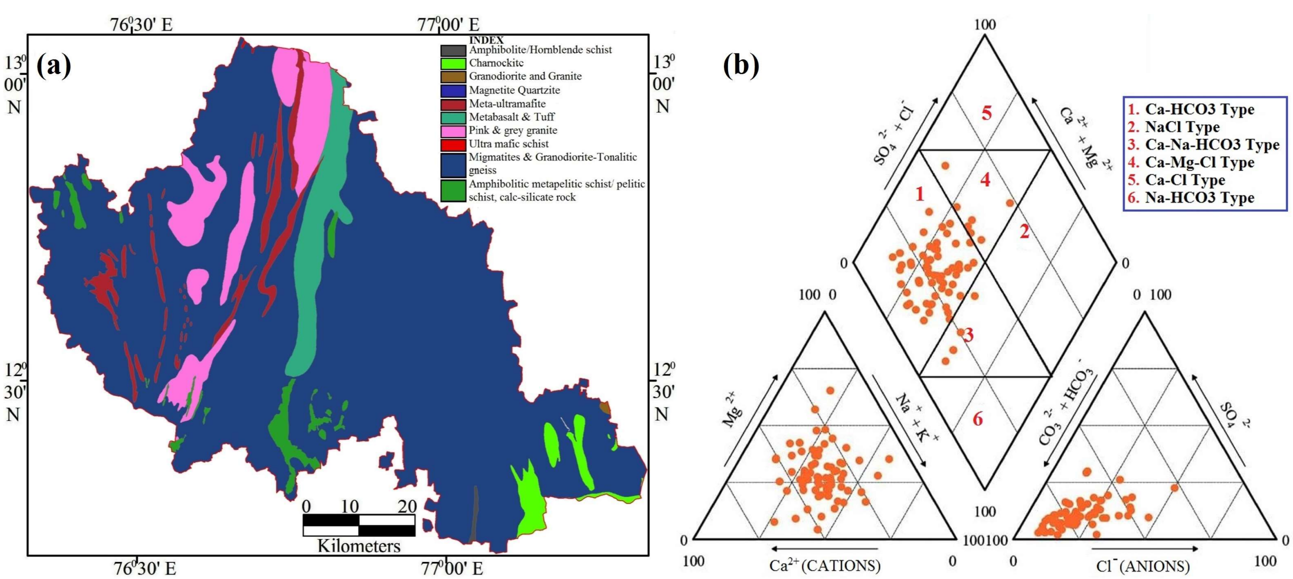

Fig. 2(a). Lithology map of Mandya district; **(b)**Piper's diagram showing hydrogeochemical facies montmorillonite, aragonite, illite, and chlorite (Basavarajappa et al., 2015c). The calcium content of the groundwater is due to the dissolution of $CaCO_3$ and $CaMg\ (CO_3)_2$ precipitates during recharge (Lakshmanan et al., 2003). (HCO_3+SO_4) vs (Ca+Mg) scatter plot reveals that silicate weathering causes ion concentration to decrease below equiline (Fig. 3c). Carbonate weathering is responsible for the concentration exceeding the equiline. The concentration drops along the equiline was caused by both silicate and carbonate weathering (Batabyal and Gupta 2017). (HCO_3+SO_4) vs (Ca+Mg) scatter diagram (Fig. 3c) of the study area indicated that most of the dots are along the equiline, with a few below and above it. This suggests that both silicate and carbonate weathering occur, with silicate weathering being more prevalent than carbonate weathering (Xiong et al., 2022). In the scenario, rainwater combines with atmospheric carbon dioxide to generate carbonic acid, which then reacts with calcium carbonate in the soil to form bicarbonate and calcium ions (Batool et al., 2024). Magnesium concentrations ranged is 6.4 to 111.6 mg/l.

Sodium and potassium: Sodium is the predominant cation in the study area that ranged from 13.69 to 5.15 mg/l. The 1:1 ratio between Na and Cl implies halite breakdown and increased Na concertation, with Cl interpreted as Na released from silicate weathering (Fig. 3a & 3b) (Mayback, 1998; Deutsch 1997). The concentrations of some samples falling below the 1:1 line of the Na vs Cl scatter diagram revealed silicate weathering, whereas concentrations above the 1:1 line of the Na vs Cl scatter diagram (Fig. 3d) shows no halite dissolution occurs. Increased concertation HCO_3 compared to Na concertation in groundwater indicated silicate weathering. Na vs HCO_3 scatter plot (Fig. 4b) showed HCO_3 concentration is high than Na, indicating silicate weathering. This implies that ion exchange mechanism diminished the Na concentration in groundwater and the same process was also supported by Na vs Ca scatter plot (Fig. 3e). The Na+K vs Total Cation scatter plot was evaluated the cation input to groundwater due to silicate

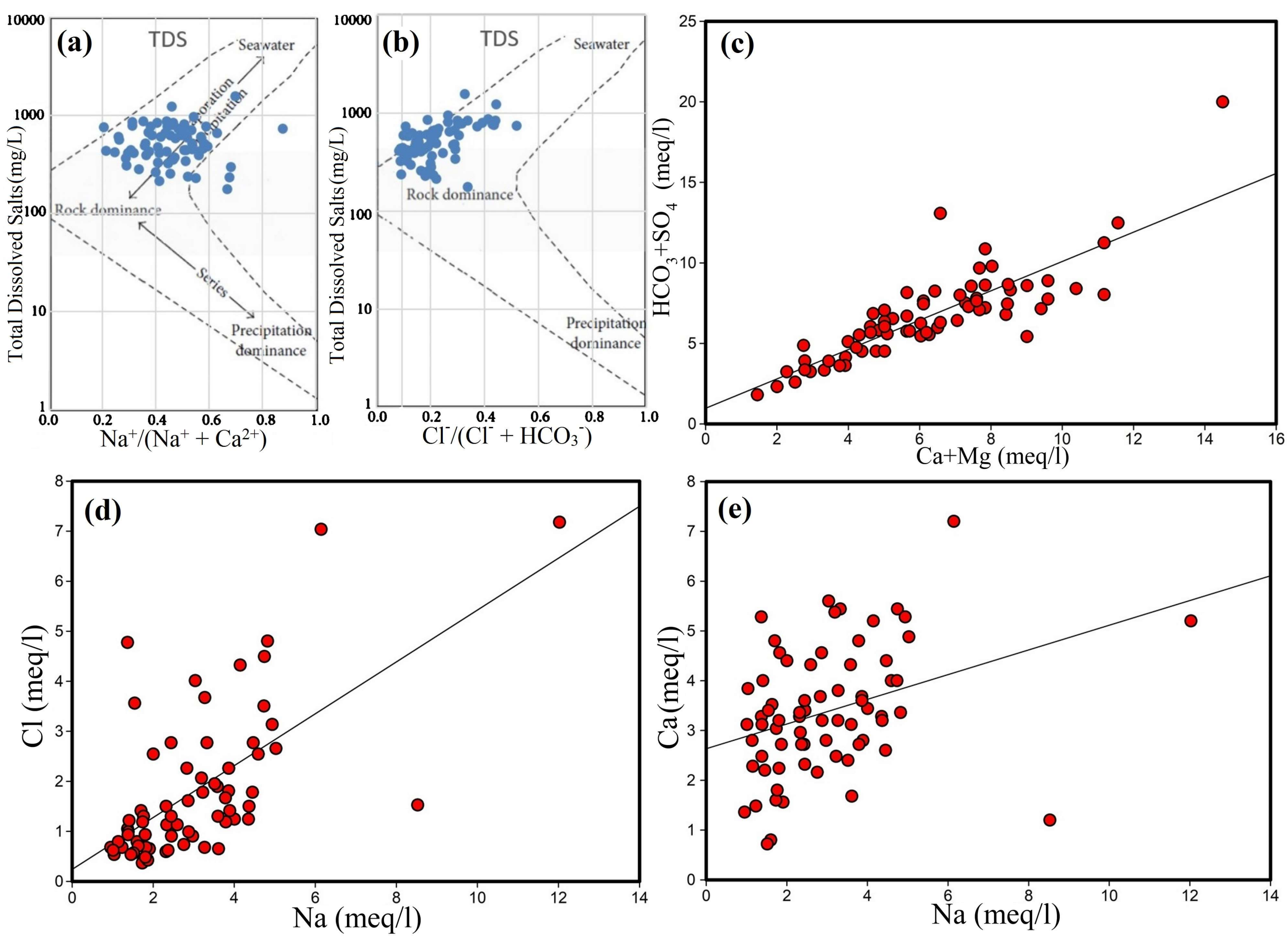

Fig. 3 (a. b) Gibb's Diagram showed the Rock-Water interactions and Scatter plots of © Ca+Mg vsHCO_3+ SO_4; **(d)** Na vs Cl; **(e)** Na vs Ca weathering. The Na+K vs Total Cation scatter map (Fig.4a) highlighted the points both above and below equiline that show cation in groundwater caused by silicate weathering. Potassium concentrations ranged from 0.28 to 73.91 mg/l.

Chloride, bicarbonate and sulphate: Bicarbonates was the predominant anion and ranged from 94.9 to 900.3 mg/l. When rainfall occurs, it combines with atmospheric carbon-dioxide to generate carbonic acid, which enters the earth and dissolves carbonate minerals. During recharge, HCO_3 and Ca will be released into groundwater. The Na vs HCO_3 concentration scatter plot (Fig. 4b) illustrated the silicate weathering increased the HCO_3 concentration in groundwater. Chloride concentrations observed to be derived from rainwater and ranged from 13 to 254 mg/l. Evaporation increased the chloride concentration in groundwater, as showed by the EC vs Na/cl scatter plot (Fig. 4c). The concentration of sulphate ranged from 6 to 251 mg/l. There is no acid rain noticed in Mandya, hence the sulphate formed via gypsum dissolving. The concentration of SO_4 vs Cl scatter diagram (Fig. 4d) revealed a low concentration of sulphate, indicating that sulphate is being depleted through sulphate reduction. As a result, sulphate concentrations in groundwater were low due to sulphate reduction or a lack of a source (Miao et al., 2012).

The main industries at Mandya are sugar mill, sugar units, jiggery producing units, rice, oil and solvent extract units (Solomon 2011). The sugar factory is one of the biggest in the nation. Chemical and Paper mill at Belagola near to Mandya town, Milk dairy at Gejjalagere, BPL battery factory and several small-scale industries were also noticed. Other major industry includes garments, textiles and engineering based. The rural population of Mandya district constituted more than 80% who involved in the vast agricultural and irrigational activities (CGWB 2012). Both industrial and agricultural activities had attributed to more use of fertilizers, pesticides, chemicals in canal irrigation.

The groundwater chemistry was influenced by the geology of the water-bearing formations of Dharwar Schist

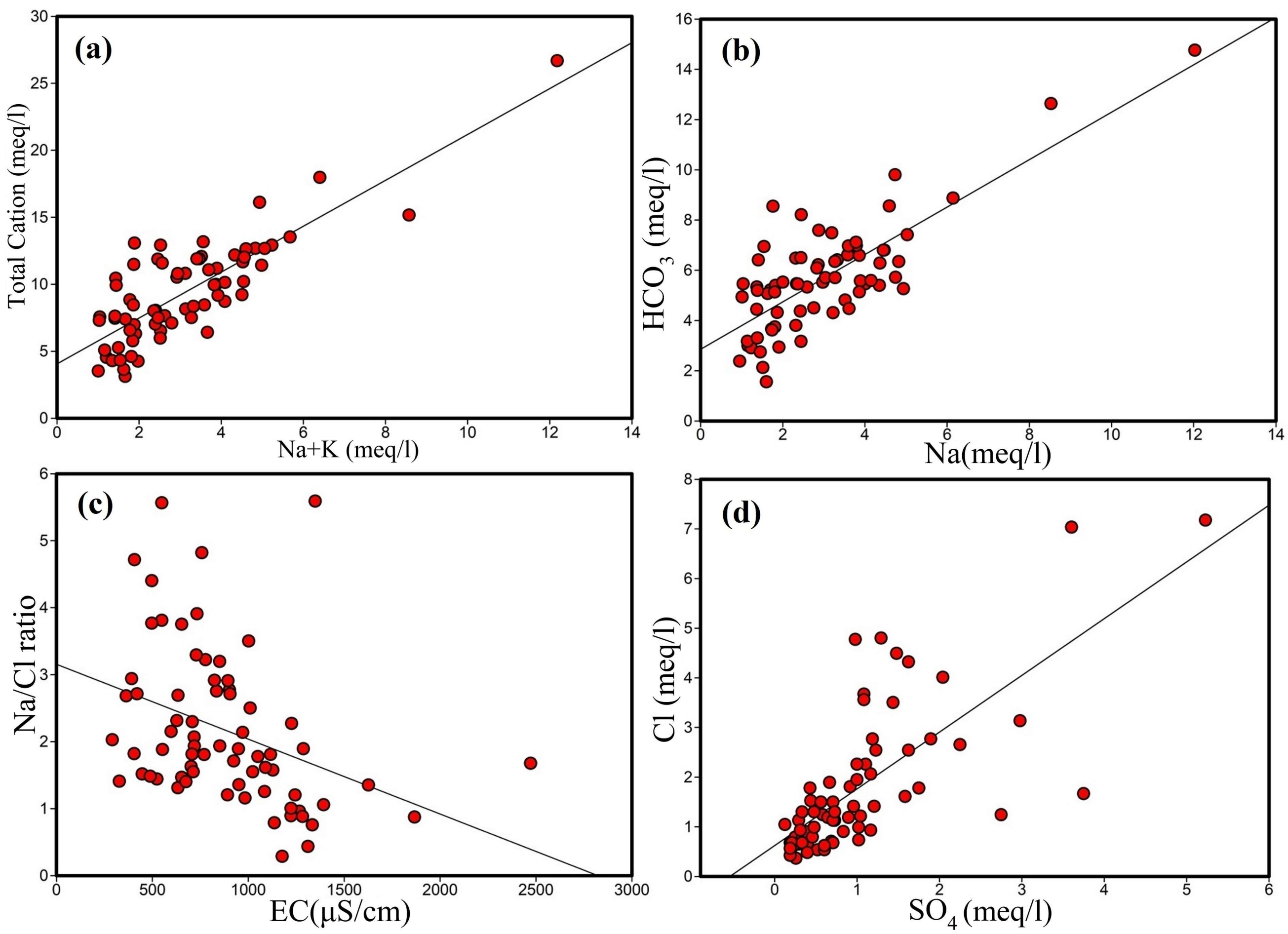

Fig. 4. Scatter plots for **(a)** Na+K vs Total cation; **(b)** Na vs HCO_3; **(c)** EC vs Na/Cl indicated Evaporation; **(d)** SO_4 vs Cl showed Sulphate reduction

and peninsular gneisses/ granites (Prasanna Kumar and Nagaraju 2000). Mandya groundwater was supersaturated with minerals like gibbsite, goethite, hematite, aragonite, calcite, dolomite, and alunite due to rock-water interactions (Yousif and El-Aassar 2018). Geoelectrical and hydrochemical studies reveal that the ions in the groundwater mostly originate from rock-water interaction, with a few samples showing precipitation dominance (Sandeep et al., 2023).

The dissolution of minerals like $CaCO_3$ and weathering of rocks were the main sources for major and trace elements leading to higher concentrations of ions like sodium, calcium, magnesium, and potassium (Yousif and El-Aassar 2018). Higher total dissolved solids (TDS) in the groundwater were attributed to the longer residence time of water in the subsurface and rock-water interaction processes (Prasanna Kumar and Nagaraju 2000). High chloride concentrations were observed in eastern part of Mandya and around Maddur. Gibbs plots indicate that groundwater samples in both seasons in Mandya has an interaction between the lithological units and the percolating water into the subsurface (Shivashankara et al., 2016).

CONCLUSION

Mandya is in Precambrian hard rock terrains with extended rock-water interaction and serve as the primary source of groundwater chemistry. Bicarbonates, calcium, and sodium were the dominant ions recorded in the collected water samples. The study region revealed three dominant hydrogeochemical faces such as Ca-Mg-Cl, Ca-Na-HCO_3, and Ca-HCO_3. This includes calcium-carbonate dissolution, silicate weathering, and ion exchange. These processes regulate groundwater chemistry contributed to a better understanding of the hydrogeochemical properties of Mandya aquifers. The rock-water interactions, aquifer geology, mineral dissolution, weathering, and seasonal recharge patterns were the key factors influencing the groundwater chemistry and quality in Mandya region. Continuous monitoring and management of these processes is important for sustainable groundwater use in the region.

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Study on Diversity of Rotifers in High Altitude Water Sources

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Abstract: The study was conducted on rotifer diversity flourishing in some lotic and lentic water sources of district Doda of Jammu and Kashmir. From the lotic water bodies, a total of fifteen species and from lentic waters, seventeen rotifer species were enlisted. Dominance of rotifer *Lepadella ovalis* in lotic waters and *Lecane closterocerca* in lentic waters was observed. Three rotifer species namely *Keratella serrulata, Notholca labis* and *Synchaeta oblonga* were recorded for the very first time in lentic water bodies of Doda district.

Keywords: Zooplankton, Habitat preference, Bio-indicator, Trophic status

Zooplankton are an integral part of any aquatic ecosystem and play a pivotal role in food web. Diversity and distribution of zooplankton is governed by number of biotic and abiotic factors and thus, they act as bio-indicator of health of aquatic system (Singh et al., 2013). Among zooplankton, rotifers are an important group and species diversity of rotifers is indicative of the trophic status of water body.

The phylum Rotifera, comprises of small microscopic organisms with average size varying from 100-1000 µm but few may acquire size of 2000µm. These comprise an important component of zooplankton and are cosmopolitan in distribution, usually free living herbivores, predators and few are parasitic (Wallace et al., 2015). These are a sensitive group of micro-organisms with diverse morphological types and are important as first food of fish larvae. These organisms are mostly free-living (swimming or crawling) but many are sessile. Majority are solitary and few are colonial as well (Wallace and Snell 2001).

Although the contribution of rotifers is small compared to total zooplankton biomass, because of small size; yet they play important part in the ecosystem of an aquatic system. These microorganisms have high ingestion rates (biomass consumed per animal per unit time) and thus, have high assimilation efficiency. Rotifers reproduce rapidly and their abundance and species composition often reflects the trophic status of a water system, and thus act as indicators (Parmar et al., 2016, Loveson et al., 2020). Rotifers form an important hinge in the aquatic food web, being prey for insect larvae, cladocerans, copepods, planktivorous fish and in turn are feed on loose periphytons, detritus, algae and small plankton.

Rotifer diversity and distribution chiefly depends upon the environmental conditions of water body such as availability of food, presence of predators or abiotic factors and therefore the distributional pattern of rotifers is not even. Such qualitative and quantitative unevenness in distribution of rotifers is also seen along altitude. Various researchers from other areas of Jammu and Kashmir has tried to study rotifer diversity Achers (Jamila et al., 2017, Kour et al., 2022) but very less work on rotifer diversity has been undertaken from high altitude areas like Doda.

Thus, keeping in view their unique body architect, importance in fisheries, cosmopolitan distribution and their role as water quality indicators, an attempt has been made presently to record the diversity and distribution of rotifers in the Doda district of Jammu province, J & K.

MATERIAL AND METHODS

Study site: Doda district falls between 32°53' and 34°21' North latitude and 75°1' and 76°47' East longitude. On northern side lies district Anantnag of Kashmir, on southwest and south are Udhampur, Kathua of Jammu and Chamba areas of Himachal Pradesh. On east and south east is Leh district. Altitude varies from 841 to 4341 m above msl. The total of five lotic (Chinta, Neeru, Bhadarwah nallah, Puldoda and Banihal) and five lentic water bodies (Lake I, Lake II, Lakr III, Fish pond Lingai and Sarkoot) from Doda district were surveyed for collection of rotifers.

Sample collection: The collection of rotifers was done at all the stations by filtering 50 litres of water through plankton net (bolting silk, 25µm mesh) during morning hours. The stations which had marginal vegetation the collection was made by vigorously shaking vegetation before filtering, in order to detach the organisms. The filtrate was collected in 20 ml plastic bottles and for preservation of rotifers 5% formalin was added.

Abiotic parameters: Data was collected on six parameters (air and water temperature, dissolved oxygen, free carbon dioxide, calcium and pH) following standard methods by (APHA 1992).

Qualitative estimation of rotifers: Identification of rotifers was done by scanning under microscope in laboratory and identification was done by following various sources and identification keys (Pennak 1978, Adoni 1985, Battish 1992, Edmonson 1992). The confirmation of rotifer species was done by study of their mastax which was exposed using Sodium hypochlorite.

Qualitative estimation of rotifers: The quantitative estimation of rotifers was done as

$$\frac{\text{Number of organisms per drop x Volume of concentrated sample in ml}}{\text{Volume of original sample x Volume of one drop (ml)}}$$

$$\textbf{Frequency} = \frac{\text{Total number of quadrants in which species occurred}}{\text{Total number of Quadrants}} \times 100$$

$$\textbf{Relative frequency} = \frac{\text{Frequency of the species}}{\text{Sum of frequency of all the species}} \times 100$$

$$\textbf{Density} = \frac{\text{Total number of species individuals of the species}}{\text{Total number of quadrants studies}}$$

$$\textbf{Relative density} = \frac{\text{Density of the speciesX100}}{\text{Sum of Density of the species}} \times 100$$

$$\textbf{Abundance} = \frac{\text{Total number of individuals of the species}}{\text{Total number of quadrants in which species occurred}}$$

Margalef' s index (1968)

$$D = \frac{S-1}{\text{Log (n)}}$$

Species diversity ($\overline{H}$)

$$\overline{H} = -S\ (P1)\ \log 2\ pi$$
$$i=1$$

where,

H= information content of sample (bits/individuals)

S= Number of Species.

P= Proportion of total species belonging to 1 species.

RESULTS AND DISCUSSION

In order to investigate the rotifer fauna of Doda district, 5 lotic systems were investigated and 15 different rotifer species (*Lepadella ovalis, Trichocerca rattus, Colurella obtusa, Colurella uncinata, Philodina* sp., *Cephalodella gibba, Euchlanis dilatata, Lecane conspicua, L. closterocerca, Trichotria tetractis, Mytilina ventralis, Polyarthra vulgaris, Testudinella patina*, *Trichocerca stylata* and *Brachionus calyciflorus*) belonging to 11 families were recorded from them (Table 1). Maximum species diversity index among these lotic systems was from Chinta stream whereas, Chenab flowing through Banihal station had complete absence of rotifers for the period of investigation. *Lepadella ovalis* as was most frequent species in these lotic waters. Maximum abundance was also shown by *Lepadella ovalis* (Table 2).

There were 5 perennial lentic waters, investigated in this district from which 17 rotifer species (*Notholca labis*, *Lepadella ovalis*, *Colurella obtusa*, *Colurella uncinata*, *Cephalodella gibba*, *Philodina* sp., *Synchaeta oblonga*, *Brachionus* sp., *B. rubens*, *B. quadridentata*, *B. patulus*, *Lecane* sp.*, L. closterocerca*, *L. curvicornis, L.* (*M*) *bulla, Keratella serrulata* and *K. tropica*) belonging to 6 families of 2 orders Ploima and Bdelloidea were recorded (Table 3). The maximum species diversity index was for fish pond. *Keratella serrulata, Notholca labis, Synchaeta oblonga* are the first report from these waters. *Philodina* sp. and *Lepadella ovalis* were the most frequently encountered rotifers in these lentic waters of Doda district. *Lecane closterocerca* showed maximum density and also maximum abundance (Table 4).

Comparatively, higher species diversity index was in lentic waters than in lotic waters presently investigated in Doda district, as lentic water sources have more stable

Table 1. List of rotifers from five lotic water bodies of Doda district

Stations	Species	n/l
Chinta	*Lepadella ovalis*	0.12
	Trichocerca rattus	0.10
	Colurella obtusa	0.08
	Philodina sp.	0.18
	Cephalodella gibba	0.04
	Euchlanis dilalatata	0.12
	Trichotria tetractis	0.06
	Mytilina ventralis	0.04
	Lecane closterocerca	0.16
	Lecane conspicua	0.14
		8.653
Neeru (7.142)*	*Colurella uncinata*	0.16
	Lecane closterocerca	0.24
	Lecane conspicua	0.04
	Philodina sp.	0.14
	Lepadella ovalis	0.10
	Trichotria tetractis	0.10
	Trichocerca rattus	0.06
Bhaderwah nallah (4.255)*	*Polyarthra vulgaris*	0.44
	Lepadella ovalis	0.20
	Testudinella patina	0.08
	Trichocerca stylata	0.08
	Brachionus calyciflorus	0.14
Puldoda	*Lepadella ovalis*	0.04
Banihal (0.00)*	-	-

*Marglef's index

environment than lotic waters in hilly terrian. Physico-chemical parameters in the lotic waters had well marked differences and showed a range of temperature from 7°C to 21°C, of dissolved oxygen from 4 mg/l to 7.2 mg/l, of calcium from 8.8 mg/l to 16 mg/l, of pH from 6.5 to 8.4 and free carbon-dioxide remained absent at all lotic stations presently studied. In lentic systems, temperature ranged from 7°C to 25°C, dissolved oxygen from 1.2 mg/l to 6 mg/l, free carbon-dioxide was 2 mg/l, calcium from 2.4 mg/l to 37.6 mg/l and pH from 6.4 to 6.9 (Table 5). These variability's in water parameters shows a well marked influence on rotifer diversity and distribution as in each and every water body, whether lotic or lentic, has different composition of rotifers. Moreover, the frequency, density, abundance of same species recorded from different water bodies was also variable. This suggests that there is a direct or indirect impact of prevailing abiotic conditions on community structureof rotifers in presently

Table 3. List of rotifers from five lenic water bodies of Doda district

Stations	Species	n/l
Lake	*Keratella serrulata*	0.04
Lake II	*Notholca labis*	0.04
(81.39)*	*Lecane* sp.	0.02
Lake III	*Brachionus* sp.	0.02
Fish pond	*Lepadella ovalis*	0.20
(Lingai)	*Cephalodella gibba*	0.12
(81.39)*	*Lecane closterocerca*	0.24
	Lecane curvicornis	0.16
	Colurella uncinata	0.06
	Colurella obtusa	0.18
	Philodina sp.	0.22
	Synchaeta oblonga	0.04
Sarkoot	*Brachionus rubens*	0.16
(69.76)*	*Brachionus quadridentata*	0.18
	Brachionus patulus	0.04
	Lecane (M) bulla	0.14
	Keratella tropica	0.10
	Philodina sp.	0.14
	Lepadella ovalis	0.06

*Marglef's index

Table 2. Various rotifer species of lotic water bodies

Species	Frequency	Relative frequency	Density	Relative density	Abundance
Philodina sp.	40	8.69	0.064	9.93	0.160
Lecane closterocerca	40	8.69	0.080	12.42	0.200
Lecane conspicua	40	8.69	0.036	5.59	0.090
Cephalodella gibba	20	4.34	0.080	12.42	0.040
Colurella uncinata	20	4.34	0.032	4.96	0.160
Colurella obtusa	20	4.34	0.016	2.48	0.080
Lepadella ovalis	80	17.39	0.092	14.28	0.250
Brachionus calyciflorus	20	4.34	0.028	4.34	0.140
Euchlanis dilatata	20	4.34	0.024	3.72	0.120
Trichocerca rattus	40	8.69	0.032	4.96	0.080
Trichocerca stylata	20	4.34	0.016	2.48	0.080
Testudinella patina	20	4.34	0.016	2.48	0.080
Trichotria tetractis	40	8.69	0.032	4.96	0.080
Mytilina ventralis	20	4.34	0.008	1.24	0.040
Polyarthra vulgaris	20	4.34	0.088	12.66	0.040

H = Shannon and weaver's diversity index 3.036

Table 4. Statistical data of various rotifer species of lenic water bodies

Species	Frequency	Relative frequency	Density	Relative density	Abundance
Philodina sp.	40	10.526	0.18	0.176	0.18
Lecane closterocerca	20	5.263	0.24	0.235	0.24
Lecane (M) bulla	20	5.263	0.14	0.137	0.14
Lecane curvicornis	20	5.263	0.16	0.157	0.16
Lecane sp.	20	5.263	0.02	0.019	0.02
Cephalodella gibba	20	5.263	0.12	0.117	0.12
Colurella uncinata	20	5.263	0.06	0.058	0.06
Colurella obtusa	20	5.263	0.18	0.176	0.18
Lepadella ovalis	40	10.526	0.13	0.127	0.13
Brachionus patulus	20	5.263	0.04	0.039	0.04
Brachionus quadridentata	20	5.263	0.18	0.176	0.18
Brachionus rubens	20	5.263	0.16	0.157	0.16
Brachionus sp.	20	5.263	0.02	0.019	0.02
Synchaeta oblonga	20	5.263	0.04	0.039	0.04
Keratella tropica	20	5.263	0.10	0.098	0.10
Keratella serrulata	20	5.263	0.04	0.039	0.04
Notholca labis	20	5.263	0.04	0.039	0.04

H = Shannon and weaver's diversity index 3.041

Table 5. Physico-chemical parameters in different water bodies of Doda district

Stations	Atmospheric temperature (°c)	Water temperature (°c)	Dissolved oxygen (mg/l)	Free carbondioxide (mg/l)	Calcium (mg/l)	pH
Lentic						
Lake I	20.2	11.0	6.0	2	3.2	6.5
Lake II	20.2	9.0	1.6	2	3.2	6.4
Lake III	20.2	9.0	2.0	2	2.4	6.4
Fish pond (Lingai)	25.0	19.0	4.4	2	8.8	6.6
Sarkoot	7.0	12.0	1.2	2	37.6	6.9
Lotic						
Chinta	20.0	18.0	7.2	-	12.0	6.8
Puldoda	21.0	17.0	6.8	-	16.0	7.2
Banihal	10.0	7.0	6.0	-	8.8	8.4
Neeru	21.0	18.0	4.0	-	12.0	6.7
Bhaderwah nallah	21.0	18.5	4.4	-	12.8	6.5

studied water bodies. This correlation of abiotic conditions and rotifer diversity is documented in earlier studies (Langer et al., 2007, Sharmila and Rajeshwari 2015, Manickam et al., 2018) .

CONCLUSION

The rotifers exhibited notable differences in various aquatic biotopes. The maximum diversity of rotifers was in water bodies with aquatic macro vegetation at littoral ends than in water systems without vegetation. This richness among vegetation is because vegetation provides food, shelter (both from light and predators) to these micro-creatures. The lentic waters showed comparatively higher rotifer diversity and richness as compared to lentic water system due to their stable environment. The present work added to the diversity of regional aquatic fauna of district Doda with first time record of three rotifer species from this high altitude area.

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Effectiveness of Processing Techniques on the Retention and Bioavailability of Nutrients in Sorghum, *Sorghum bicolor* (L.) Moench

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Abstract: The effect of various processing techniques namely soaking, blanching germination, roasting, puffing, decortication and milling on nutritional composition retention and bioavailability of nutrients of sorghum (PSC 4) was assessed. Puffing, soaking and germination resulted in a significant increase in the protein (6.8, 7.4 & 9.4% respectively) and dietary fiber (4.4%) while roasting and puffing significantly enhanced *in vitro* starch digestibility of starch (27.8-33.5%). Bioactive compounds like phenols (11.1-26.0%) and flavonoids (14.5-28.0%) were significantly reduced by processing while germination increased its antioxidant activity (16.7%). However, iron content was significantly increased by roasting and puffing (14.4-17.9%) while calcium and zinc content was reduced with processing applications. Since, germination and puffing showed affirmative relationship with retention of nutrients with enhanced starch, protein and mineral absorption, therefore, these techniques can be regarded as the most viable applications for household processing and are rather instrumental in popularizing sorghum as a sustainable substitute of staple cereals with enhanced nutrition to combat food and nutritional security.

Keywords: Germination, Puffing, Parling, Dietary fiber, Iron, Zinc, Bioavailability

As the world is currently experiencing climate change and ever-increasing burden of population, the coming decades might see a decline in the crop production. The proportion of less fertile soils is predicted to increase by 50 to 56 per cent with 78 per cent of it affecting developing countries. Besides, an increase in the cultivation of crops like rice, maize, sugarcane etc. requiring extra water is responsible for a loss of approximately 7191 litres of ground water per hectare (Kumar et al., 2018). Research showed that cultivation of millets also plays a distinct role in decreasing atmospheric carbon dioxide, thus contributing constructively to climate change (Saxena et al., 2018). Among these, Sorghum, *Sorghum bicolor L. moench* requires soil salinity of 4-6 dS/m and rainfall required for optimum maturity (90-120 days) is 40-100 cm (Fageria et al., 2010). However, other research studies suggest that seedlings of some of the genotypes of sorghum are capable of surviving high soil surface temperature up to 40^{o}C (Nguyen et al., 2013). These grains score better over rice and wheat in terms of amino acid profile, dietary fiber and a great virtue of nutrients including iron, folate, calcium, zinc, magnesium, phosphorus, copper, vitamins, antioxidants and bioactive compounds.

Sorghum grains are loaded with starch, cellulosic and non-cellulosic polysaccharides (mainly glucuronoarabinoxylans [GAX]). Thus, they have somewhat high gelatinization temperature leading to lower starch digestibility. Addition of sorghum bran to the diet may help protect against development of metabolic disease states such as obesity, type II diabetes, and inflammation by improving colonic microbiota (Lloyd et al., 2016). Being a gluten-free cereal and a chief source of a bundle of nutrients, sorghum is a good dietary substitutes for celiac disease patients. Since sorghum is not available in convenient food forms besides their coarse nature, low digestibility and presence of anti-nutritional factors such as phenolic compounds, these nutri-grains lost their popularity with the transforming world. Sorghum has low protein bioavailability due to presence of anti-nutritional factors like trypsin inhibitors, tannin, and phytic acid and it lacks essential amino acids like methionine, lysine, and isoleucine.

Some conventional processing techniques are applied prior to preparation and consumption, such as soaking, decorticating, germination, malting and fermentation which tend to improve nutritive, and sensory characteristics of the millets (Jaybhaye and Srivastav 2015). In addition, improved bioavailability of nutrients and reduced anti-nutritional factors are the benefits of processing. Malting tends to improve bioavailability of iron by increasing its absorption up to 300 per cent while manganese absorption is increased by 17 per cent (Platel et al., 2010). Germination causes hydrolysis of phytate phosphorus to form inositol monophosphate causing decrease in phytic acid, thus reducing its phytate content (Handa et al., 2017). Soaking followed by germination helps in leaching tannins out of millet grains (Hussain et al., 2011).

Fermentation improves protein digestibility by reducing anti-nutrient load. Germination and fermentation increase the antioxidants characteristics and reduces the phytate content of sorghum and pearl millet (Kayode et al., 2013). Keeping in view the benefits of these nutri-cereals in health and disease, the present study attempts to assess the nutritional aptitude such as retention and bioavailability of nutrients by application of various processing techniques.

MATERIAL AND METHODS

Sample preparation: Sorghum cultivar (PSC 4) sourced from the Seed Production Farm of Punjab Agricultural University, Ludhiana, (sown in May 2017) was cleaned and subjected to following treatments (Table 1).

Control (unprocessed grains) and processed sorghum grains were dried in a hot air oven (Model MSW 211, Macro Scientific Works Pvt. Ltd., India) to achieve 5 % moisture content and finely ground using mortar and pestle. Samples were stored in labelled, airtight containers. All chemical reagents used were of analytical grade and reagent kits were purchased from Sigma-Aldrich, Inc. Sigma Chemical Co. (USA).

Determination of proximate composition: Moisture (method 44–10.01, AACC 2010) and ash (gravimetrically by charring then igniting at 550 °C in a muffle furnace using method 08-01.01, AACC 2010) was measured. Crude fat was analysed (920.39, AOAC 1997) using SOCS plus Solvent Extraction system (Pelican, India) while crude protein using Kjeldahl method (46-13.01, AACC 2010) in KEL PLUS Automatic Nitrogen System (Pelican Equipment, Chennai, India) with a factor of 6.25 applied to convert the amount of nitrogen to crude protein. The carbohydrate content was calculated by subtracting sum of all proximate parameters (moisture content, crude protein, crude fat, crude fiber, and total ash) from 100. Total dietary fiber was quantified using a modified enzymatic gravimetric method (991.42, AOAC 2005).

Dietary fiber: The soluble, insoluble and total dietary fiber contents of the samples were analyzed in triplicates using Megazyme-K-TDFR- 200A. The soluble and insoluble dietary fiber contents were analyzed using the standard protocol given by AOAC (1997).

Bioactive Compounds and Antioxidant Activity

Bioactive compounds: Total phenolic content was determined by method described by (Singleton et al., 1999) whereas tannins was estimated by modified method of Owheruo et al. (2018). The determination of flavonoid content was performed using a method followed by Zhischen et al (1999) as elucidated by Owheruo et al. (2018) and measured as mg rutin equivalent per 100g dry weight.

Antioxidant activity was estimated by following two methods: Total antioxidant activity by DPPH was measured following the methodology stated by Brand-Williams et al. (1995) and total antioxidant activity by FRAP was measured according to method by Benzie and Strain (1999).

***In vitro* digestibility:** *In vitro* starch digestibility was analysed using methodology by Sharma et al. (2018) and protein digestibility as per the methodology of Sharma et al. (2018).

Minerals analysis: Iron, calcium and zinc were estimated using atomicabsorption spectrophotometer (Analyst 2000, Perkin Elmer, USA) post wet digestion (Merwe et al., 2019).

***In vitro* mineral bioavailability:** For iron the methodology stated by Rao and Prabhavati (1978) was used. For calcium and zinc bioavailability methdology specified by Rebellato et al. (2020) was used.

Statistical analysis: Data so obtained was expressed as mean with standard deviations, analysed for one-way analysis of variance and post hoc test (Tukey's) and the means separated using Least Significant Difference at $p = 0.05$, performed using IBM SPSS Inc. (version 23, Chicago, USA).

RESULTS AND DISCUSSION

Proximate composition: As represented in Table 2, the crude protein content was significantly ($p \leq 0.05$) varied among control and processed flour of sorghum PSC-4 (SOR). The highest and lowest crude protein content of control and processed SOR was detected in puffed and parled grains' flour, respectively with a range varying between 8.09 to 9.86 percent with comparable increase by soaking (9.68 g/100g) and germination (9.62 g/100g). Similar increase in protein content of fermented cereals was also reported by Tian et al. (2010) in their study attributing to microbial synthesis of protein during the process of germination (Adebiyiet al., 2017). It was also observed in the literature that increase in germ size of grains by processing is responsible for the increase in its protein content (Singh and Raghuvansi 2012). However, the fat content of milled and parled SOR flour was observed to be significantly different from all other processed SOR flours. The fat content of control and processed sorghum flour varied between 1.90 to 4.00 per cent with highest content in parled flour and lowest in germinated flour. Our results were in agreement with the findings reported by Mohapatra et al. (2018) who revealed reduction in fat content post fermentation of sorghum grains from 4.7 per cent to 3.6 per cent. The low-fat content of processed flours can contribute to increased shelf-life due to decreased probability of rancidity but at the stake of reduced energy value. Nevertheless, the crude fiber content of germinated SOR grain flour was found to be significantly ($p \leq 0.05$) higher (1.91 g/100g) as compared to control and other processed SOR grain flours while parled SOR flour had lowest (0.81 g/100g) content. This observation was accredited to the removal of bran layer during decortication process, thus reducing the fiber content of the grain. Our finding corroborated the findings of Fasasi (2009) who reported the crude fiber content of germinated pearl millet flour to be 1.80 per cent attributing to the utilization of sugar for energy to perform metabolic activities like sprouting while leaving the fibrous content in the grain.

Total dietary fiber: The dietary fiber content of control, blanched, puffed and milled SOR flours were found to be significantly ($p \leq 0.05$) different from their other processed contemporaries (Table 2). It ranged between 7.33 to 8.57 per cent with highest content pertaining to germinated flour and lowest in parled flour. Germinated (8.57%) and soaked (8.53%) SOR flour was found to have significantly ($p \leq 0.05$) higher dietary fiber. The observed reduction may be attributed to the removal of germ and pericarp during parling process. Our findings were in accordance with the results reported by Pushparaj and Urooj (2011) who revealed a reduction in dietary fiber content (9.2%) post partial removal of bran layer as compared to whole flour (13.3%) while the dietary fiber content of germinated flour was reported to be higher (13.4%) than whole flour. Spike during germination could be due to structural disruption of polysaccharides in grain's cell wall possibly affecting the anatomical intactness of tissue and hampering the carbohydrate-protein interaction leading to extensive biosynthesis of new cell wall and producing new dietary-fiber (Sharma et al., 2015). Increase in puffed variant could be due to increase in β-glucan

Table 1. Processing of sample

Treatment	Method
Germination	Grains were soaked in water (1:2) overnight (30℃) and treated with formaldehyde (0.2 %) tailed by washing and incubating in muslin cloth at 30°C. Germination was done for 48 h (90–95% RH) and dried to a constant weight at 50℃ in a hot air oven.
Soaking	The cleaned grains of pearl millet were soaked overnight at room temperature (25℃) in excess distilled water in a dish, covered with a muslin cloth for 6 h.
Blanching	Blanching was done as per procedure described by Chavan and Kachare (1994). According to this method, distilled water was brought to boiling temperature of 98℃. The sorghum grains were subjected to blanching in boiling water (1:5 ratio of seeds to boiling water) for 30 seconds and then dried at 50℃ for 60 minutes.
Roasting	Application of dry heat at 190℃ and removed from the flame immediately after the roasted nutty flavour begin to arise from the pan of grains (after 3 min).
Puffing	By the method of Malleshi and Desikachar (1981), moisture content was raised to 19 % and puffed in an iron frying-pan using fine sand as a heat exchange medium (270°C). Puffed sample was separated by sieving through 40 mesh sieve.
Milling	Procured sorghum grains were milled by mechanical method using a laboratory hammer mill (Relitech Industries, Gujarat, India) using no. 0 stainless steel sieve (particle size < 70µm).
Parling	Grains were parled for one minute by mechanical method using 'Barley Parler Control Unit' with approximately 8-9 % removal of bran (decortication).

Table 2. Effect of various treatments on nutritional composition (%) of sorghum (dry matter basis)

Crop	Treatment	Moisture	Ash	Crude protein	Crude fat	Crude fiber	Carbohydrates	Total dietary fiber (%)	Energy (KCal)
SOR	C	5.09^{a}±0.27	3.08^{a}±0.06	9.01^{ab}±0.53	2.46^{a}±0.58	1.37^{a}±0.16	78.36^{ab}±0.33	8.17^{ab}±0.32	374.14^{ab}±3.80
	T1	9.01^{b}±1.21	1.22^{b}±0.67	9.62^{a}±0.30	1.90^{a}±0.05	1.91^{ab}±0.03	77.02^{a}±1.61	8.57^{b}±0.32	363.63^{a}±7.68
	T2	6.08^{ac}±2.39	1.41^{b}±0.12	9.68^{a}±0.36	2.47^{a}±0.08	1.21^{a}±0.08	79.15^{ab}±2.68	8.53^{b}±0.39	377.54^{b}±10.24
	T3	5.78^{a}±0.53	1.29^{b}±0.03	8.68^{ab}±0.25	2.42^{a}±0.17	1.30^{a}±0.05	83.86^{c}±0.30	7.97^{ab}±0.25	391.91^{c}±1.30
	T4	2.58^{d}±0.04	1.28^{b}±0.03	8.65^{ab}±0.26	2.49^{a}±0.11	1.22^{a}±0.39	83.78^{c}±0.28	8.37^{b}±0.45	392.15^{c}±1.06
	T5	4.28^{ad}±0.03	1.57^{bc}±0.06	9.86^{a}±0.27	2.35^{a}±0.19	1.31^{a}±0.10	80.63^{bc}±0.40	7.87^{ab}±0.31	383.07^{bc}±1.09
	T6	5.20^{a}±0.06	2.34^{d}±0.05	9.05^{ab}±0.82	2.97^{ab}±0.92	1.26^{a}±0.08	79.19^{ab}±0.88	7.87^{ab}±0.42	379.64^{bc}±4.62
	T7	8.29^{c}±0.03	2.21^{cd}±0.52	8.09^{b}±0.58	4.00^{b}±0.96	0.81^{c}±0.06	76.60^{a}±1.86	7.33^{a}±0.40	374.75^{ab}±3.67

Mean values followed with different subscripts are significantly different ($p \leq 0.05$) using Tukey's test for different parameters (moisture, ash, crude protein, crude fiber, carbohydrates, total dietary fiber and energy).

PM-1: Pearl millet FBC 16; PM-2: Pearl millet PCB 165; SOR: Sorghum PSC 4

C- control sorghum/ pearl millet flour, T1-germinated sorghum/ pearl millet flour, T2- soaked sorghum/ pearl millet flour, T3- blanched sorghum/ pearl millet flour, T4- roasted sorghum/ pearl millet flour, T5-puffed sorghum/ pearl millet flour, T6-milled sorghum/ pearl millet flour, T7-parled sorghum/ pearl millet flour

availability accounting to release of bound β-glucan due to thermal effect (Kora 2019).

Bioactive Compounds and Antioxidant Activity

Total phenols: Total phenol content of control and processed flours were expressed as mg gallic acid equivalent per 100g sample (Table 3). A significant ($p \leq 0.05$) rise of 10 per cent in the total phenol content was found in milled SOR flour as compared to control. Total phenols of control and processed SOR flour ranged between 105.18 to 156.51mg GAE/100g with the highest content exhibited in milled SOR flour and lowest in its blanched counterpart. All processing treatments except milling resulted in a significant decrease in the total phenol content when compared to control. According to Taylor and Duodo (2015), phenolic compounds of sorghum grains are concentrated in the outer layers, therefore, removal of bran during decortication could be possible cause for their reduction in flour. As well as the reduced phenolic compounds due to germination can be accredited to the leaching of phenolic compounds in the steeping liquid owing to its enhanced solubilization. Also, the drifting of phenols from outer layers to endosperm during soaking hampers its extractability due to formation of complexes with protein and other major molecules, might be another reason for reduction in total phenol content during soaking and germination. Oxidation and thermal degradation of phenols during thermal processing like roasting and puffing could be the consequence of its reduced content than control. It was also reported that post-harvest treatment had a negative impact on retention of total phenols as conjugated polyphenolics degrades to simpler compounds (Kadiri 2017). Another study by Zhang et al., (2010) reported a significant decline in total phenols of buckwheat flour post thermal processing.

Tannins: The tannin content of control and processed sorghum flour had a range between 25.30 to 35.22 mg/100g with highest content in control flour and lowest in germinated and flour. Research evidence revealed migration of condensed tannins to the endosperm along with imbibed water during steeping and germination. Taylor and Duodo (2015) also stated that tannin extractability of sorghum flour could be reduced post germination owing to the formation of irreversible complexes with its kafirin prolamin protein. Although, prolonged germination may cause reverse migration of tannins towards the outer layers and enhance its extractable content as in case of malting (Kayodé et al., 2007). Additionally, production and catalytic effect of enzymes (esterases) due to sprouting tend to hydrolyse the tannins, thus causing its reduction in the produced flour (Chethan et al., 2008).

Total flavonoid content: The total flavonoid content of control and processed samples were expressed as 'mg rutin equivalents per 100g sample' (Table 3) and analysis pronounced that the processed SOR flours except puffed variant displayed a significant ($p \leq 0.05$) decline in the total flavonoid content in comparison to control flour. Range of total flavonoids of control and processed SOR flours lied between 68.96 mg RE/100g in the parled flour to 110.87 mg RE/100g in the control fraction. The lowest content being observed in the parled variant can be owed to the removal of outer bran layer in the process of parling, where these bioactive compounds are concentrated the most. The observation was also in line with the findings reported by Sreeramaiah and Goudar (2012) who observed a substantial reduction in the total flavonoid content in flaked sorghum grains. This decline was owed to the pre-processing applications like soaking, removal of bran and boiling before final pressing by the authors.

Total Antioxidant Capacity by DPPH (1,1 diphenyl-picrylhydrazyl/2,2-Diphenyl-1-picrylhydrazyl): Radical

Table 3. Effect of various treatments on bioactive compounds and antioxidant activity of and sorghum (dry matter basis)

Crop	Treatment	Total phenols (mg GAE/100g)	Tannins (mg/100g)	Total flavonoids mg RE/100g	DPPH TAC (mg TE/100g)	FRAP TAC (mg TE/100g)
SOR	C	142.21^{ab}±2.65	35.22^{a}±4.33	110.87^{a}±4.19	422.97^{a}±16.05	241.90^{a}±12.41
	T1	109.53^{c}±12.16	25.26^{b}±3.01	100.51^{ab}±2.89	493.54^{b}±7.34	356.77^{b}±1.07
	T2	126.47^{ac}±7.26	34.56^{a}±2.65	89.62^{b}±2.74	426.64^{a}±8.87	246.16^{a}±3.24
	T3	105.18^{c}±12.16	32.54^{a}±2.34	95.59^{b}±8.00	363.02^{c}±11.26	263.53^{a}±23.79
	T4	117.54^{ac}±15.86	33.89^{a}±4.79	96.44^{b}±2.37	350.67^{cd}±3.59	251.52^{a}±3.21
	T5	118.29^{ac}±5.92	32.80^{a}±3.04	109.46^{a}±3.03	498.67^{b}±9.74	320.09^{c}±14.80
	T6	156.51^{b}±13.98	30.10^{c}±5.51	100.05^{ab}±5.19	442.28^{a}±14.72	250.34^{a}±4.24
	T7	111.09^{c}±4.86	25.30^{b}±2.96	68.96^{c}±1.66	327.37^{d}±7.86	161.61^{d}±2.39

Mean values followed with different subscripts are significantly different ($p \leq 0.05$) using Tukey's test for different parameters [Total phenols, tannins, total flavonoids, total antioxidant activity (DPPH & FRAP)].

PM-1: Pearl millet FBC 16; PM-2: Pearl millet PCB 165; SOR: Sorghum PSC 4

C- control sorghum/ pearl millet flour, T1-germinated sorghum/ pearl millet flour, T2- soaked sorghum/ pearl millet flour, T3- blanched sorghum/ pearl millet flour, T4- roasted sorghum/ pearl millet flour, T5-puffed sorghum/ pearl millet flour, T6-milled sorghum/ pearl millet flour, T7-parled sorghum/ pearl millet flour

scavenging activity (Table 3) was studied by DPPH (1,1 diphenyl- picrylhydrazyl/2,2-Diphenyl-1-picrylhydrazyl) for control and processed fractions sorghum PSC 4 (SOR). Total antioxidant capacity of all the analysed samples was expressed as 'mg trolox equivalent/100g'. Statistically significant ($p \leq 0.05$) increase in the antioxidant activity was detected in germinated (493.54 mg TE/100g) and puffed (498.67 mg TE/100g) SOR flour. Although, blanching (363.02 mg TE/100g), roasting (350.67 mg TE/100g) and decortication (327.37 mg TE/100g) were found to significantly ($p \leq 0.05$) reduce the total antioxidant activity of the SOR flour. Revelation of comparable results was done by Singh et al., (2019) who noted a significant enhancement in the antioxidant activity post germination. The authors also demonstrated a positive correlation between duration of germination and its antioxidant activity (% inhibition of DPPH). The reported antioxidant activity rose from around 12 percent after twelve hours to about 40 percent at the end of 48 hours. Significant ($p \leq 0.05$) rise in the radical scavenging activity (by DPPH) post germination can be attributed to the induction of high levels of enzymes (superoxide-dismutases, glutathione-S-transferase, peroxidises and catalases) with antioxidative properties (Gupta et al., 2013).

Higher antioxidant activity in puffed variant can be due to the existence of higher total phenolic content in the flour. This statement was justified by Alothmanet al. (2009) who had defined direct correlation between percent DPPH inhibition and total phenols. The authors also attributed this outcome to the increased formation of Millard compounds due to high temperature exposure in a short time period. Additionally, the observation of the present study was in agreement with the findings reported by Pradeep and Guha (2011) that heat treatment was observed to increase the antioxidant activity of little millet by 95.5 percent as compared to germination which increased it by 91.7 percent.

Total Antioxidant Capacity by Ferric Reducing Antioxidant Power (FRAP)

Analysis of total antioxidant capacity by Ferric Reducing Antioxidant Power (FRAP) was expressed as 'mg Trolox equivalent/ 100g' (Table 3). Germination, puffing and decortication significantly ($p \leq 0.05$) affected the antioxidant power when analysed for FRAP. Germination and puffing significantly ($p \leq 0.05$) enhanced the antioxidant power by 47.5 and 32.3 percent respectively while decortication reduced it significantly ($p \leq 0.05$) by 33.2 percent owing to the removal of outermost bran layer holding phenols, flavonoids and antioxidants. Lowest to highest FRAP values of control and processed SOR flour ranged between 241.90 mg TE/100g in control to 356.77 mg TE/100g in germinated flour.

***In vitro* protein and starch digestibility:** The term *in vitro* suggests imitating a metabolic process outside of a living organism while the *in vitro* digestibility of nutrients account for the amount of nutrients absorbed and metabolized once ingested. The effect of processing treatments on *in vitro* protein and starch digestibility has been documented in Table 4. The *in vitro* protein digestibility of control and processed sorghum PSC 4 ranged between 49.81 to 56.44 percent with lowest digestibility was observed in blanched sorghum flour and highest in its germinated counterpart with a non-significant difference between them. This observation can be accredited to a myriad of hydrolytic enzymes released during processing especially due to germination. These enzymes have the potential to hydrolyse biopolymers (storage proteins) making it easily available for pepsin hydrolysis and it also degrades the anti-nutritional factors, thus, making the nutrients highly digestible.

Amid in vitro starch digestibility, sorghum PSC 4 flour displayed a significantly ($p \leq 0.05$) higher starch digestibility in roasted (42.03 mg maltose released per gram) and puffed (43.91 mg maltose released per gram) variants as compared to control (32.89 mg) while a significantly ($p \leq 0.05$) low digestibility was observed in blanched flour (27.57 mg maltose released per gram). This observation was however in line with the results reported by Roopa and Premavalli (2008) who revealed that puffing grains significantly improved the starch digestibility of finger millet. The results obtained in present investigation were in concordance with a study by Huang et al. (2018) who reported significant

Table 4. Effect of various treatments on dietary fiber and *in vitro* nutrient digestibility of sorghum (dry matter basis)

Crop	Treatment	*In vitro* starch digestibility (mg maltose released/g	*In vitro* protein digestibility (%)
SOR	C	$32.89^{a}\pm1.31$	$51.12^{a}\pm18.24$
	T1	$37.77^{a}\pm0.90$	$56.44^{a}\pm7.98$
	T2	$35.78^{a}\pm1.83$	$51.42^{a}\pm1.42$
	T3	$27.57^{b}\pm1.67$	$49.81^{a}\pm10.56$
	T4	$42.03^{c}\pm2.48$	$52.74^{a}\pm8.80$
	T5	$43.91^{c}\pm0.29$	$50.05^{a}\pm1.71$
	T6	$34.22^{a}\pm3.38$	$53.41^{a}\pm8.47$
	T7	$34.34^{a}\pm1.22$	$55.06^{a}\pm2.85$

Mean values followed with different subscripts are significantly different ($p \leq 0.05$) using Tukey's test for different parameters (*in vitro* nutrient digestibility).

PM-1: Pearl millet FBC 16; PM-2: Pearl millet PCB 165; SOR: Sorghum PSC 4

C- control sorghum/ pearl millet flour, T1-germinated sorghum/ pearl millet flour, T2- soaked sorghum/ pearl millet flour, T3- blanched sorghum/ pearl millet flour, T4- roasted sorghum/ pearl millet flour, T5-puffed sorghum/ pearl millet flour, T6-milled sorghum/ pearl millet flour, T7-parled sorghum/ pearl millet flour

increase in starch digestibility post puffing grains, owing to enhanced rate of hydrolysis probably due to starch gelatinization. Research evidence also described that higher degree of gelatinization create prospects for enzymes (amylases) to attack the starch causing its hydrolysis (Mishra et al., 2014).

Total Minerals and their *in vitro* Bioavailability

Iron: Analysis of control and processed SOR flour revealed variant results (Table 5). It was observed that milling (6.67 mg/100g), puffing (6.37 mg/100g), roasting (6.18 mg/100g) and germination (5.75 mg/100g) significantly ($p \leq 0.05$) improved the iron content of SOR flour (5.40 mg/100g) while blanching (4.86 mg/100g) and soaking (4.82 mg/100g) significantly ($p \leq 0.05$) reduced the content. Research analysis also reported a significant increase in mineral extractability of pearl millet between 23 to 70 percent post application of domestic processing like germination. This observation could owe to the reduction in phytic acid contents which otherwise would make complexes with minerals leaving it unabsorbed (Coulibalyet al., 2011).

Calcium and zinc: Calcium content of SOR flour was observed to reduce significantly ($p \leq 0.05$) post processing application from 21.46 to 10.88 mg/100g (Table 6). The lowest content was observed in parled (10.88 mg/100g) flour due to removal of outer bran and germ which holds these minerals. The least reduction in calcium content of SOR flour was observed in its milled variant (20.02 mg/100g). However, other processing treatments had calcium content in order of germination > puffing > roasting > soaking > blanching (19.23, 19.17, 18.60, 18.17, 16.87 mg/100g, respectively). Similar

Table 5. Effect of various treatments on total and bioavailable iron of and sorghum (dry matter basis) (calculation)

Crop	Treatment	Iron		
		Total (mg/100g)	Percent Ionizable iron at pH 7.5 (X)	*In vitro* iron bioavailability (Y) (% iron absorption in adults)
SOR	C	$5.40^{a}\pm0.29$	$2.37^{ab}\pm0.46$	$1.60^{ad}\pm0.22$
	T1	$5.75^{ab}\pm0.29$	$3.76^{bc}\pm0.68$	$2.25^{bc}\pm0.32$
	T2	$4.82^{c}\pm0.06$	$2.15^{a}\pm0.32$	$1.50^{ad}\pm0.15$
	T3	$4.86^{c}\pm0.20$	$2.83^{ab}\pm0.55$	$1.82^{abd}\pm0.3$
	T4	$6.18^{bd}\pm0.07$	$4.21^{c}\pm0.57$	$2.46^{c}\pm0.26$
	T5	$6.37^{d}\pm0.13$	$2.97^{bc}\pm0.71$	$1.88^{bcd}\pm0.34$
	T6	$6.67^{d}\pm0.14$	$2.36^{ab}\pm0.34$	$1.59^{ad}\pm0.16$
	T7	$4.13^{e}\pm0.33$	$1.99^{a}\pm0.47$	$1.42^{ad}\pm0.22$

Mean values followed with different subscripts are significantly different ($p \leq 0.05$) using Tukey's test for different parameters (total and bioavailable iron).

PM-1: Pearl millet FBC 16; PM-2: Pearl millet PCB 165; SOR: Sorghum PSC 4

C- control sorghum/ pearl millet flour, T1-germinated sorghum/ pearl millet flour, T2- soaked sorghum/ pearl millet flour, T3- blanched sorghum/ pearl millet flour, T4- roasted sorghum/ pearl millet flour, T5-puffed sorghum/ pearl millet flour, T6-milled sorghum/ pearl millet flour, T7-parled sorghum/ pearl millet flour

Table 6. Effect of various treatments on total and bio available calcium and zinc of pearl millet and sorghum (dry matter basis)

Crop	Treatment	Calcium			Zinc		
		Total (mg/100g)	Bioavailable (mg/100g)	Percent bio-availability	Total (mg/100g)	Bioavailable (mg/100g)	Percent bio-availability
SOR	C	$21.46^{a}\pm0.67$	$5.23^{abc}\pm0.56$	$24.35^{a}\pm1.81$	$3.12^{a}\pm0.17$	$0.24^{abd}\pm0.03$	$7.62^{ab}\pm0.39$
	T1	$19.23^{bc}\pm0.38$	$5.69^{bc}\pm0.31$	$29.61^{ab}\pm2.65$	$3.26^{a}\pm0.04$	$0.30^{b}\pm0.02$	$9.16^{b}\pm0.61$
	T2	$18.17^{d}\pm0.22$	$4.93^{abc}\pm0.35$	$27.14^{ab}\pm1.71$	$2.51^{bc}\pm0.07$	$0.19^{ac}\pm0.02$	$7.50^{ab}\pm0.81$
	T3	$16.87^{e}\pm0.31$	$4.20^{ad}\pm0.46$	$24.93^{a}\pm3.13$	$2.58^{c}\pm0.06$	$0.14^{c}\pm0.05$	$5.33^{a}\pm2.00$
	T4	$18.60^{bd}\pm0.33$	$4.57^{abd}\pm0.42$	$24.53^{a}\pm1.85$	$2.63^{c}\pm0.13$	$0.21^{acd}\pm0.07$	$8.06^{ab}\pm0.51$
	T5	$19.17^{bc}\pm0.34$	$5.51^{ab}\pm0.47$	$28.78^{ab}\pm1.48$	$2.31^{bd}\pm0.04$	$0.21^{acd}\pm0.01$	$9.14^{b}\pm0.47$
	T6	$20.02^{c}\pm0.32$	$4.87^{abc}\pm0.61$	$24.34^{a}\pm2.26$	$3.85^{a}\pm0.19$	$0.28^{bd}\pm0.01$	$7.36^{ab}\pm0.39$
	T7	$10.88^{f}\pm0.16$	$3.57^{d}\pm0.31$	$32.81^{b}\pm3.13$	$2.18^{d}\pm0.06$	$0.19^{ac}\pm0.06$	$8.80^{ab}\pm2.91$

Mean values followed with different subscripts are significantly different ($p \leq 0.05$) using Tukey's test for different parameters (total and bioavailable calcium and zinc).

PM-1: Pearl millet FBC 16; PM-2: Pearl millet PCB 165; SOR: Sorghum PSC 4

C- control sorghum/ pearl millet flour, T1-germinated sorghum/ pearl millet flour, T2- soaked sorghum/ pearl millet flour, T3- blanched sorghum/ pearl millet flour, T4- roasted sorghum/ pearl millet flour, T5-puffed sorghum/ pearl millet flour, T6-milled sorghum/ pearl millet flour, T7-parled sorghum/ pearl millet flour

revelation was observed in a study by Afify et al., (2012) who reported significant reduction in the calcium content in three sorghum varieties post soaking (26.74, 18.90 and 16.51 mg/100g), cooking (13.15, 16.74 and 14.58 mg/100g) and germination (12.50, 12.50 and 18.79 mg/100g) when compared to their control (33.09, 26.59 and 22.91 mg/100g) counterparts. Significantly ($p \leq 0.05$) higher absorption of available calcium was observed in parled (32.81%) SOR flour, followed by germinated (29.61%) SOR flour. Other processing treatments also proved to significantly ($p \leq 0.05$) enhance the absorption percentage of calcium content in the order of puffing > soaking > blanching > roasting > milling (28.78, 27.14, 24.93, 24.53, 24.34% respectively).

Total zinc content was also observed to reduce significantly ($p \leq 0.05$) by application of processing treatment to SOR flour owing to leaching in soaking medium. The zinc content of control and processed SOR flour ranged between 2.18 mg/100g in parled flour and 3.26 mg/100g in germinated SOR flour. Afify et al. (2012) also reported the zinc content of processed (soaked, cooked and germinated) sorghum flours to range between 3.12 mg/100g and 3.78 mg/100g. The observation of the above-mentioned study validated the observation of the present study. A significant ($p \leq 0.05$) enhancement in the percent bioavailability was observed in processed SOR flours as compared to control flour. Maximum zinc absorption was established by germinated (9.16%) and puffed (9.14%) SOR flours followed by decortication, roasting, soaking and milling (8.80, 8.06, 7.50 and 7.36 % respectively). However, percent bioavailability of zinc was significantly reduced in blanched (5.33%) SOR flour.

CONCLUSION

The major constraint for wide utilization of sorghum being its low availability and acceptability attributing to its fat content and undesirable flavour can be countered by applying conventional processing. Germination and puffing considerably retained and increased the crude protein and total dietary fiber, respectively while reducing the fat content. *In vitro* starch digestibility was improved by processing too. Iron content improved substantially by thermal processing while calcium and zinc were lost by heat treatments and parling. However, germination and puffing showed affirmative relationship with iron, calcium and zinc absorption. Therefore, germination and puffing resulted in desirable nutritional characteristics of sorghum cultivar and these treatments at household level are instrumental in popularizing these grains for value addition and enhanced nutrition and can be regarded as the most viable applications for household processing to combat food and nutritional security.

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