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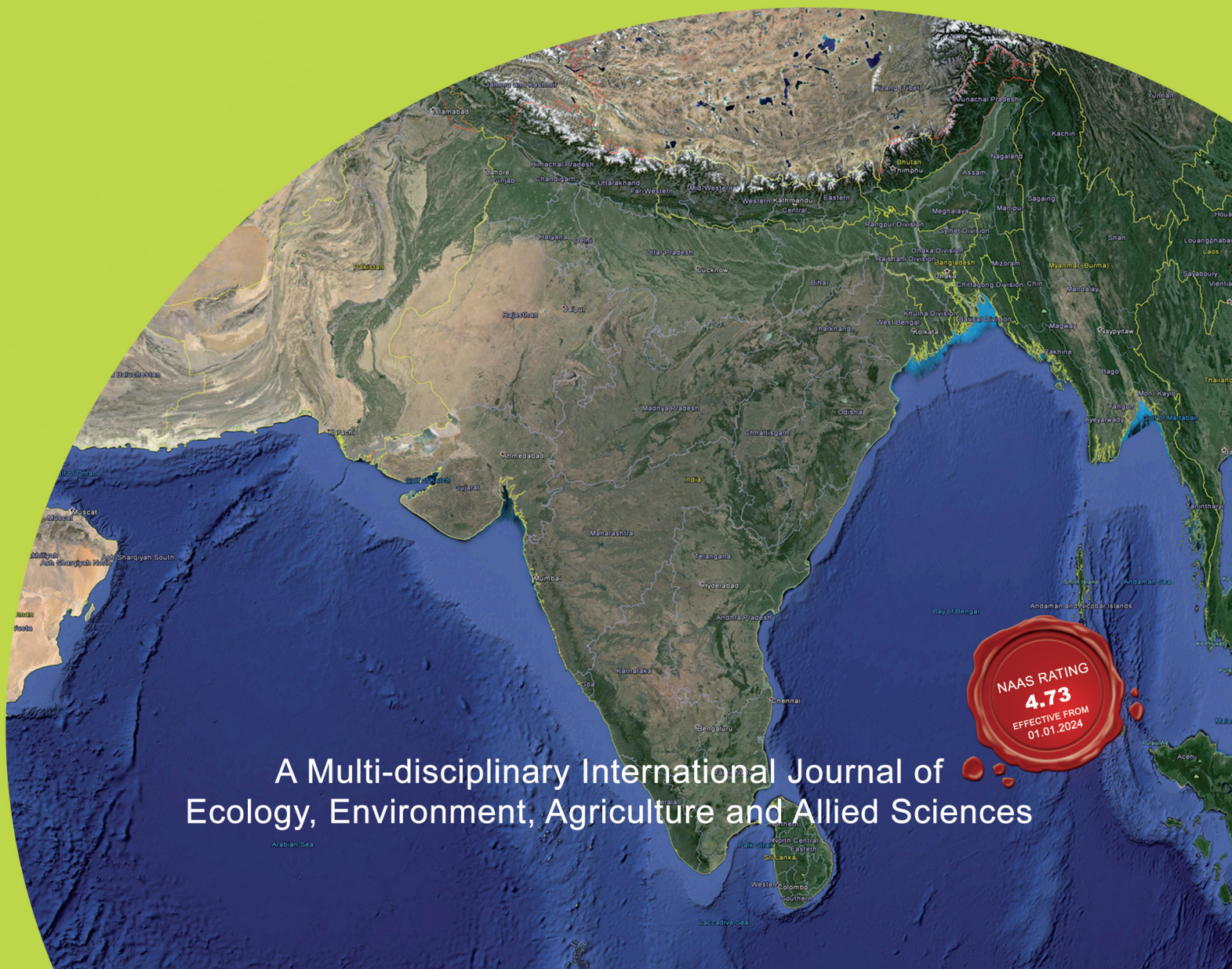

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ADDRESS FOR CORRESPONDENCE

Dr. R.K. Samantarai
Editor-in-Chief
A - 47, Rameswarpatna, Maushima Square
Bhubaneswar - 751 002, Odisha
e-mail - eplanetjournal@gmail.com
rksamantray876@gmail.com
Mob. : +91 7008370017 / 9437090017
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Nutri-sensitive agriculture in India: A comprehensive review

G.S. POOJA[1], S.P. LAL[2*] AND V.G. REDDY[3]

[1,3]*Department of Agricultural Extension Education, Jayashankar Telangana Agricultural University, Hyderabad-500030, India*

[2*]*Agricultural Technology Information Centre, Directorate of Extension Education, RPCAU, Pusa, Samastipur, Bihar- 848125, India*

**sudhanand.lal@rpcau.in*

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ABSTRACT

Nutri-sensitive agriculture (NSA) has emerged as an essential approach for achieving sustainable food systems, improved health and ecological stability. It integrates nutritional goals with agricultural production through crop diversification, dietary diversity, biofortification and environmentally sustainable practices. Although India has achieved self-sufficiency in food production with a TWR-5, total output of 353.9 million tonnes and national availability of 283.1 million tonnes, widespread malnutrition persists. Around 172.1 million people remain undernourished, and about 586.5 million individuals cannot afford a healthy diet. The prevalence of anaemia among women exceeds 50%, while among children below five years of age, 32.1% are underweight, 19.3% suffer from wasting, and 35.5% are stunted. These nutritional deficiencies highlight the urgent need to integrate nutrition-focused and environmentally responsible strategies into agricultural systems. The recent release of 152 biofortified crop varieties between 2014–2024 has been a significant step towards improving the nutritional value of staple foods, aligning with NSA objectives. The present study examines the need assessment and evolution of NSA in India by analyzing food production trends, affordability of healthy diets and nutritional outcomes from 1951 to 2025. It also describes the initiatives, policies, and interventions that have contributed to combating malnutrition since independence. The study emphasizes the role of environmental sustainability in achieving nutrition goals and suggests strategic integration of nutrition into agriculture through established frameworks of FAO, UNICEF, MSSRF and IFAD.

Key words: Biofortification, food availability, framework, nutri-sensitive agriculture, sustainability

INTRODUCTION

India, being an agrarian country, has witnessed a whopping 500% rise in food grain production since independence, boosting the overall rural economy. In 2023-24, total food grain production was estimated at 332.3 million tonnes (PIB, 2025). Food grain availability on a per capita per day basis rose from 395 g in 1951 to 638.8 in 2023 (Economic survey, 2024-25). Despite sustainably producing food grain, about 172.1 million people in India were malnourished (FAO, 2025). Over US $ 12 billion in GDP per year in India is lost to vitamin and mineral deficiencies (HarvestPlus, 2021) and India contributes a third of the global burden of malnutrition (WHO, 2023). India holds 11.1% of the world's extreme poor, a proportion that is steadily decreasing but remains the second largest in absolute numbers after Nigeria, according to recent global poverty estimates (Singh and Singh, 2018). This provides evidence of improved food availability and accessibility; however people still face food

and nutrition insecurity. However, by achieving high levels of food production, India has paved its way towards attaining food security. However, ambiguity remains regarding the achievement of comprehensive nutritional security across the country.

Food security is all about gaining or fulfilling the 4 dimensions such as availability, accessibility, utilization and stability (Gross et al., 2000; Hahn, 2000; Rötten, 2000; Weingärtner, 2009), which, when coupled with adequate food intake and good health, improves nutritional status. Nutritional Security can be defined by the availability, accessibility of diverse, nutritious food (Ruel, 2013). Thus, achieving food and nutritional security primarily depends on agriculture and integrating nutrition into agriculture that pave the way to attain nutritional security with environmental sustainability by providing nutritious and diverse foods. This has led to the emergence of nutri-sensitive agriculture (NSA). The conventional farming system is primarily focussed on food production and productivity aiming at food security while NSA aims to improve the accessibility and availability of nutrient-rich foods. NSA promotes crop diversification and sustainable intensification aimed at both productivity and environmental sustainability (FAO, 2017). NSA not only contributes in striking the nutritional deficiencies but also contributes to 12 sustainable development goal-indicators (Global Food Policy Report, 2016; Yadava et al., 2022; United Nations, 2025).

MATERIALS AND METHODS

This study employed an analytical mixed-methods approach based on the qualitative and quantitative data.The analysis spanned a long-term period from 1990 to 2025, analyzing data exclusively from selected national and international sources. The data sources include Economic Survey of India, National Family Health Survey (NFHS) of India, Indiastat, Press Information Bureau: Prime Ministers Office, India, Global Hunger Index, FAOSTAT, the State of Food Security and Nutrition in the World (SOFI) reports, jointly produced by UN agencies (IFAD, UNICEF, WFP and WHO). The data were examined to provide a holistic understanding of nutri-sensitive agriculture in India particularly focusing on the pathway toward sustainability.

RESULTS AND DISCUSSION

Food grain production and per capita availability

India's food grain production has shown a six-fold rise since independence achieving macro-level food security (PIB, 2022). Total production has more than doubled from 169.9 Mt (1988-89) to a projected 353.9 Mt in 2024-25 (Fig. 1). The growth is dominated by rice and wheat, whose combined share increased significantly, largely driven by supportive policies and technological advancements since the Green Revolution era. This staple dominance prioritizes calorific yield over nutritional diversity. Pulses (protein-rich) and coarse cereals (nutri-millets), despite recent policy focus (e.g., NFSM-Pulses, Shree Anna), show slower and fluctuating growth; pulses productivity is 3.75 times lower than that of the rice–wheat system (Srivastava and Lal, 2021). Pulses reached a projected 24.3 Mt, and nutri-millets 62.1 Mt in 2024-25. In 2002-03 there was a sharp decline in the food grain production due to a severe drought that occurred leading to a deceleration in GDP growth to 4.0 per cent in 2002-03, mainly because of a sharp decline of 5.2 per cent in the agriculture and allied sector (PIB, 2004).

Per capita food grain availability rose from 395 g/day in 1951 to 514 g/day in 2023 (Fig.2). The previous peak was recorded at 510 g/day in 1991, reflecting shifts in production and population growth. The net per capita monthly availability was approximately 17.3 kg (Singh, 2025). Pulses, an important source of protein, have shown marginal growth in per capita availability, raising concerns about balanced nutrition. However, per capita consumption of cereals has declined amid changing diets, and limited growth in pulses highlights ongoing concerns about nutritional balance despite improved overall food security facing the dual challenge of improving nutritional quality and meeting the needs of a still-growing population (Singh et al., 2023).

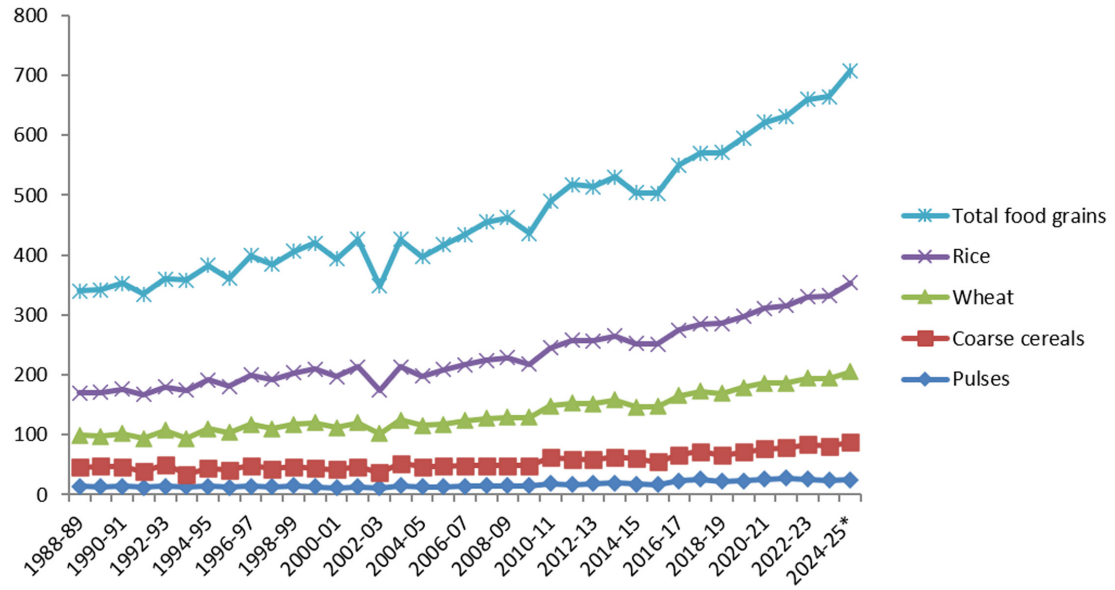

Fig. 1. Food grains production (million tonnes): 1988 - 2025

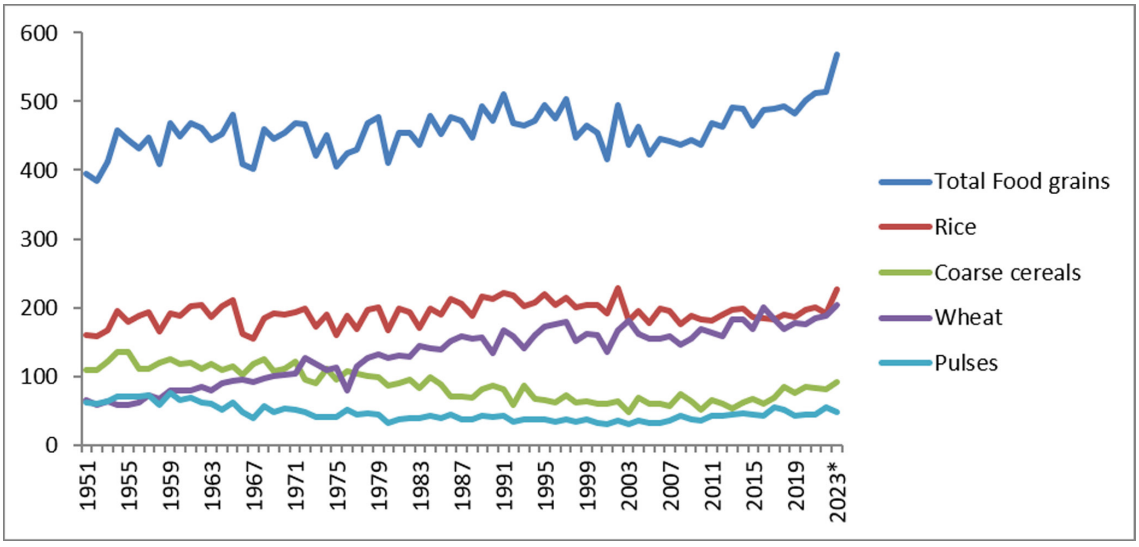

Fig. 2. Per capita availability of food grains (g/day): 1951-2023 (a quadrennial study)

Accessibility

Nearly 80.1 crore beneficiaries, including households under the Antyodaya Anna Yojana and priority households, access subsidized grains through the PDS at over 5.4 lakh fair price shops across India. India's net food grain production has steadily grown, rising from 48.1 Mt in 1951 to 295.6 Mt by 2023, accompanied by increases in net availability, which moved from 52.4 to 289.1 Mt over the same period. The share of food grains distributed through the PDS expanded from 8 Mt in 1951 to approximately 55–57 Mt annually since 2015 (Table 1), mirroring the nation's commitment to food security and inclusive access. This alignment emphasizes how policy reforms, technological adoption, and expanded irrigation have enabled consistent production gains, often following droughts and key interventions (Swaminathan and Bhavani, 2013). The significant PDS expansion, especially after the enactment of the National Food

Security Act, 2013 and the adoption of targeted subsidies, has played a vital role in supporting vulnerable groups and cushioning food security during the COVID-19 pandemic crisis (George and McKay, 2019; Pooja et al., 2023).

Table 1. Net production, net availability and public distribution of food grains in million tonnes (from 1951 - 2001 decade wise and 2001-2023 year wise)

Year	Net production of food grains (in Mt)	Net availability of food grains (in Mt)	Public distribution
1951	48.1	52.4	8
1961	72	75.7	4
1971	94.9	94.3	7.8
1981	113.4	114.3	13
1991	154.3	158.6	20.8
2001	172.2	156.9	13.2
2002	186.2	189.5	18.2
2003	152.9	170.6	23.2
2004	186.5	183.3	28.3
2005	173.6	170	31
2006	182.5	181.9	31.8
2007	190.1	183.7	32.8
2008	210.2	183.5	34.7
2009	205.2	189.5	41.3
2010	190.8	189.2	43.7
2011	213.9	203.1	47.9
2012	232.9	205.4	44.9
2013	231.9	220.6	44.5
2014	231.9	222.2	43.5
2015	220.5	213.8	53.7
2016	220.1	226.3	56.6
2017	241.7	229.8	57.8
2018	255.3	234.6	56.4
2019	255.7	235.7	56.6
2020	266.6	247.9	56.2
2021	278.4	255.3	53
2022	283.1	259.1	55.9
2023	295.6	289.1	55.8

Food grains stock under targeted public distribution system (TPDS) and other welfare schemes (OWS)

India has a massive food grain stock, equivalent to stacking bags of grain 2.347 times the distance to the moon (Lal et al., 2022). Between 2019 and 2024, food grain stocks under the Targeted Public Distribution System (TPDS) displayed fluctuations driven by procurement levels, consumption patterns, government policies, and interventions. Stocks rose from 458.2 lakh tonnes in 2019 to a peak of 568.5 lakh tonnes in 2020, maintained elevated levels through 2021, and then declined to a low of 332.1 lakh tonnes by 2023, before slightly recovering to 376.6 lakh metric tonnes in 2024 (Fig.3). The accumulation of stocks during the pandemic years (2020–21) was a strategic effort to safeguard food security, expand welfare initiatives like Pradhan Mantri Garib Kalyan Anna Yojana (PMGKAY), and manage market uncertainty. The sharp downturn after 2021 was due to higher distribution through welfare schemes, market interventions to stabilize prices, and efforts to reduce excess stocks and associated storage costs, combined with variable harvests and export restrictions. The recent increase in 2024 reflects renewed procurement and government efforts to rebuild buffer reserves. These trends underscore the role of TPDS food grain stocks in addressing both policy shifts and situational demands, ensuring food security, stabilizing prices, and supporting vulnerable populations through timely government interventions.

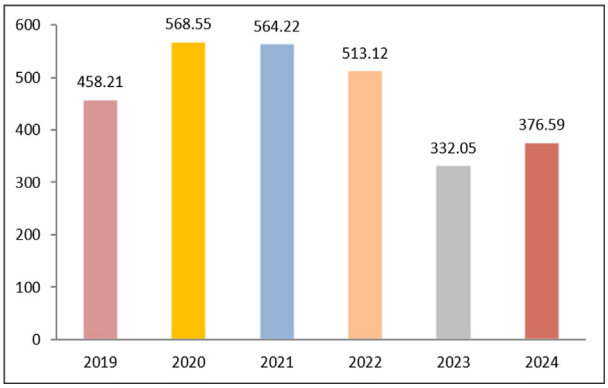

Fig. 3. Stock position of food grains under TPDS and OWS (million tonnes)

Affordability of healthy diet

The number of Indians unable to afford a healthy diet reduced from about 804.9 million in 2017 to 586.5 million in 2024. Correspondingly, India's share of the global population in this category also fell from 27.4% to 22.5% during the same period, paralleling a global decline as the worldwide tally fell from 2,934.2 million to 2,604.6 million (Table 2).

Table 2. Comparative prevalence of unaffordability of healthy diet among people of India and world

Year	India (million)	World (million)	% share of India
2017	804.9	2934.2	27.43
2018	750.3	2818.5	26.62
2019	723.1	2762.1	26.18
2020	780.2	2911.4	26.80
2021	729.4	2746.7	26.56
2022	672.5	2683.7	25.06
2023	617.2	2653.4	23.26
2024	586.5	2604.6	22.52

Despite this progress, India still accounts for over one-fifth of all people globally unable to afford nutritious diets, highlighting the persistent challenge of nutritional security. Improvements are linked to enhanced agricultural self-sufficiency, broader social protection through schemes like the National Food Security Act (NFSA), TPDS and interventions to stabilize food prices, all of which helped prevent further deprivation during COVID-19 (Kaur, 2025). Nonetheless, nearly one-fourth of the world's diet-insecure population remains in India, with continuing constraints such as income inequality, food price inflation, dietary imbalance, and uneven reach of welfare measures. Additional barriers include increased costs of nutritious foods relative to staple grains, climate shocks, erratic weather patterns, inflation, stagnant farm incomes, and declining landholdings, all of which affect dietary diversity and affordability (UN-India, 2022; Jangir and Goswami, 2025; Sushmita et al., 2025).

Nutritional health status

The National Family Health Survey (NFHS) data and FAOSTAT undernourishment statistics reflect mixed progress for nutrition and health in India. Prevalence of anaemia in non-pregnant women (15-49 years) increased from 51.8% (NFHS-2) to 57.2% (NFHS-5). Anaemia among pregnant women fluctuated, with a low of 49.7% (NFHS-2), peaking at 57.9% (NFHS-3), and stabilizing around 52.2% in NFHS-5. Anaemia rates among adolescent girls (15–19 years) rose from 54.1% in NFHS-4 to 59.1% in NFHS-5 (Fig. 6). For men (15–49 years), anaemia was lowest in NFHS-4 (22.7%), but increased to 25% in NFHS-5 (Fig .7)

The estimated number of undernourished people in India peaked at 247 million (2003–2005), declined steadily to 143.9 million (2016–2018), but rose again between 2018 and 2022, reaching 201.9 million (2020–2022) before falling to 172.1 million (2022–2024) as reflected in Fig. 4. 12% of the Indian population is undernourished. This reflects both progress during the push for food security and setbacks due to recent economic, climatic, and pandemic disruptions. Stunting prevalence declined from 51% (NFHS-2) to 35.5% (NFHS-5), while underweight decreased from 42.7% to 32.1%. Wasting rates fluctuated, peaking at 22.9% in NFHS-3, but fell to 19.3% in NFHS-5 (Fig. 5). Improvements are significant, but persistent rates remain among the highest globally, India ranks second in child wasting and twenty-first in child stunting in the Global Hunger Index, 2025.

Despite overall declines in stunting and underweight among children, the reduction rate is not sufficient to meet national and global nutrition targets, particularly after modest setbacks in recent years associated with socioeconomic and health system shocks (Mukherjee et al., 2022; PIB, 2023). Anaemia remains an acute public health challenge, especially among women and children, with NFHS-5 revealing a worsening trend compared to NFHS-4. Women's anaemia prevalence remains above 50%, and the gender gap is clear, as demonstrated by much lower rates among men (Sushmita et al., 2025; Jindal et al., 2025; Tripathi et al., 2023). The uptick in undernourished population since 2018

underscores the importance of resilient food and nutrition systems (Papola, 2025). Disruptions due to COVID-19, inflation, and uneven welfare access disproportionately affected vulnerable groups. Multi-factorial causes such as mother's education, socio-economic status, sanitation, birth order, and maternal nutritional status drive child malnutrition in India (Shah et al., 2024).

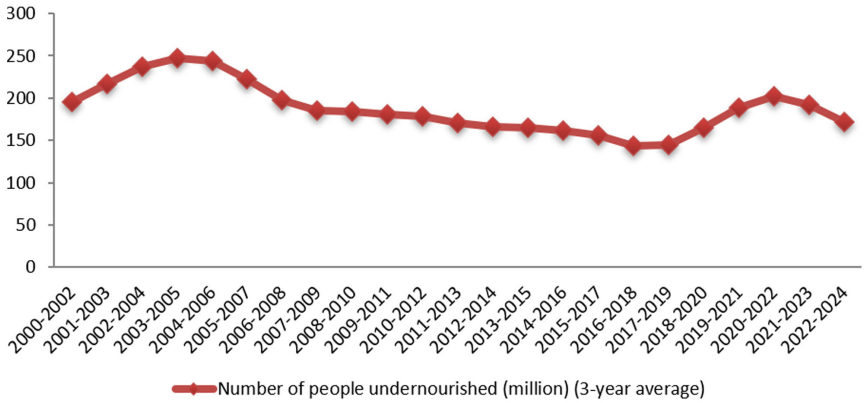

Fig. 4. Number of people undernourished in India (2000-2024)

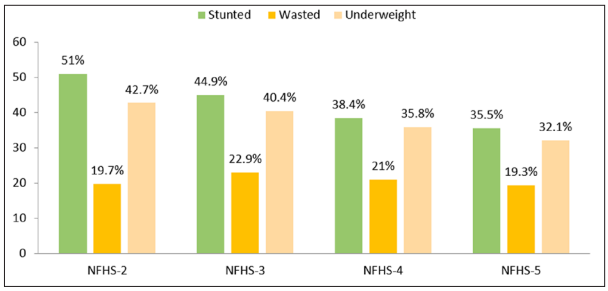

Fig. 5. Anthropometric indicators of child nutrition

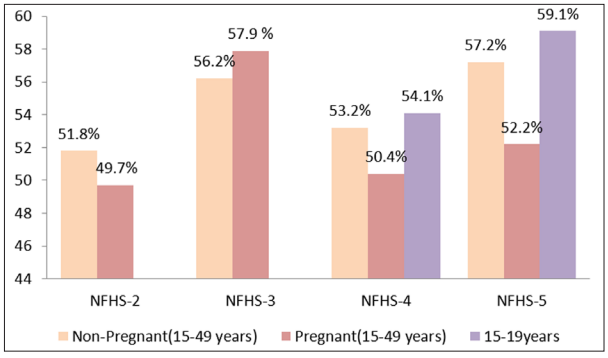

Fig. 6. Prevalence of anaemia in women

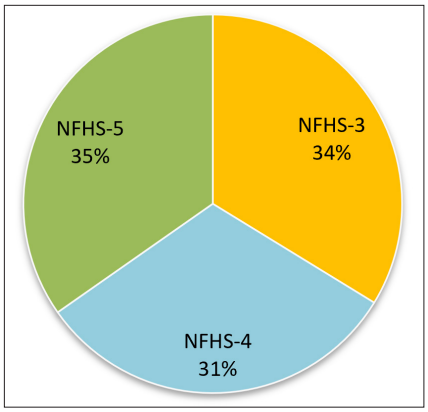

Fig. 7. Prevalence of anaemia in men

Global Hunger Index

India ranked 102nd out of 123 countries in the Global Hunger Index (GHI) 2025, with a score of 25.8, placing it in the "serious" hunger category (Table 3). The GHI score has gradually improved since 2000, but remains well above the "moderate" threshold, indicating persistent challenges to food security and nutrition.

Table 3. Global Hunger Index score of India (2000-2025)

Global Hunger Index	2000	2008	2015	2023	2025
Low					
Moderate					
Serious			29.2	28.7	25.8
Alarming	38.4	35.5			
Extremely alarming					

Schemes or initiatives in India to ensure food and nutritional security

India's approach to food and nutrition security has progressed from a focus on broad self-sufficiency to targeted nutritional interventions, beginning with the establishment of the Public Distribution System in 1947 and expanding through initiatives like Antyodaya Anna Yojana, (2000), National Food Security Act, (2013) and the major extension of Pradhan Mantri Garib Kalyan Anna Yojana (PMGKAY) in 2024 (Table 4). Nutrition-specific measures such as Integrated Child Development Services (ICDS), rice fortification, Shree Anna Abhiyan, and the diversification of crops mark a gradual shift toward enhancing diet quality and addressing micronutrient deficiencies. The rebranding of the National Food Security Mission to the National Food Security and Nutrition Mission in 2024-25 expanded its coverage to include pulses and coarse cereals, supporting comprehensive nutritional outcomes, while the Mission for Aatmanirbharta in Pulses (2025) represents a recent push for pulse self-reliance and addressing chronic dietary deficits.

Table 4. Food and nutritional security programmes in India

Year	Scheme
1947	Public Distribution System
1970-71	Balwadi Nutrition Programme
1975	Integrated Child Development Services (ICDS) Scheme
1993	National Nutrition Policy
1995	Mid-Day Meal Scheme
1999	Annapurna Scheme
2000	Antyodaya Anna Yojana (AAY)
2005	National Rural Health Mission (NRHM)
2007	National Food Security Mission (NFSM)
2007	Rashtriya Krishi Vikas Yojana (RKVY)
2010	Indira Gandhi Matritva Sahyog Yojna (IGMSY)
2011	Mahila Kisan Sashaktikaran Pariyojana (MKSP)
2013	National Food Security Act (NFSA)
2013	National Health Mission (NHM)
2015-16	Direct Benefit Transfer (DBT) in PDS
2017	National Nutrition Mission (Poshan Abhiyaan - 2018)
2017	Pradhan Mantri Matru Vandana Yojana (PMMVY)
2017	Nutri-sensitive Agricultural Resources and Innovations (NARI)
2017	Knowledge Systems for Homestead Agriculture Management in Tribal Areas (KSHAMTA)
2017	Value Addition and Technology Incubation Centers in Agriculture
2019	One Nation One Ration Card
2019	Rice Fortification Initiative
2021	Pradhan Mantri POSHAN (POshan SHAkti Nirman) Scheme
2021	Nutri-Smart Village Programme
2023	Shree Anna Abhiyan
2024	Pradhan Mantri Garib Kalyan Anna Yojana (PMGKAY)
2024-25	National Food Security and Nutrition Mission (NFSNM)
2025	Mission for Atmanirbharta in Pulses

The clear escalation in initiatives since 2014, particularly between 2014 and 2024, is highlighted by the introduction of 152 biofortified crop varieties (Fig. 8) and widespread launch of Poshan Vatikas, which enable communities to grow and access nutritious, diverse foods year-round (Kumari and Lal, 2023) with an emphasis on education and empowerment of women and children. Collectively, flagship programs like POSHAN Abhiyaan and these nutrition-sensitive agricultural interventions exemplify India's integrated, multi-level policy response, shifting decisively from simple calorie adequacy towards holistic, sustainable nutritional security.

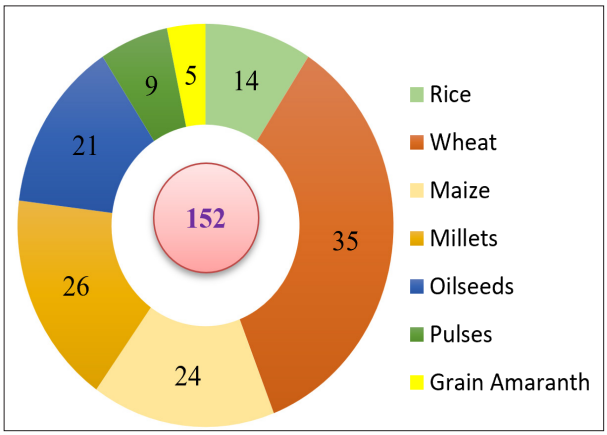

Fig. 8. Total number of biofortified varieties released during 2014 to 2024

Nutri-sensitive agriculture

Experts and agencies emphasize that accelerating progress in India's food system will require intensified nutrition-sensitive policies, greater investment in resilient local agriculture, expanded support for dietary diversity, and women's empowerment. Without this integrated approach, malnutrition risks being entrenched, impeding the country's achievement of UN sustainable development goals (UN-India, 2022; Gulati et al., 2023; Jangir, 2025; Singh, 2025). Nutrition-sensitive agriculture places nutritionally rich foods, dietary diversity, and food fortification at the core of overcoming malnutrition and micronutrient deficiencies, as advocated by FAO (2014). This concept involves integrating nutrition into agricultural policy through crop diversification, biofortification, dietary diversity, nutrition education, and gender-inclusion, aiming to improve the nutritional status of vulnerable populations It also recognizes women's critical role in food production and nutrition outcomes.

Several frameworks integrate nutrition with agriculture and health care systems that have been presented below.

UNICEF conceptual framework for under-nutrition

The UNICEF conceptual framework for undernutrition describes how a range of factors at national, community, household, and individual levels that influence nutritional status. It highlights that undernutrition has immediate causes (poor diet and disease), underlying causes (household food insecurity, inadequate care, and poor health services and sanitation), and basic causes (social, economic, and political conditions). The framework underscores that children's nutrition depends on access to nutritious food, adequate care, healthcare services, and a safe environment. These influences can trap families in cycles of illness and poverty across generations and highlight the importance of addressing nutrition early in life to prevent stunting, poor brain development, and long-term disease. Understanding these interconnected factors helps guide effective interventions to reduce undernutrition and improve public health.

Duncan et al. (2022) expanded this framework to integrate the food and agriculture sector with nutrition outcomes . The integration recognizes that the food and agriculture sector is deeply connected to underlying causes of malnutrition.The expanded framework identifies how nutritional outcomes and agriculture are linked in six important ways through evidence-based food and agriculture system components: (i) Food (ii) Income (iii) Food prices (iv) Women's empowerment (v) Women's time utilization (vi) Women's health and nutritional status. The food and agriculture sector facilitates interventions through multiple pathways such as production activities, processing activities, consumption activities and farmer practices and behaviour. This framework

helps identify intervention points for planning and implementing multisectoral nutrition programs.

Farming systems for nutrition-MSSRF

The FSN framework is designed as a comprehensive approach to address nutritional deficiencies through agriculture-based interventions. The framework was developed to explore nutrition-sensitive agricultural interventions and tested in India from 2013-2018. The model was designed with community members consultation to create a participatory approach that addresses local nutrition needs. The framework recognizes the potential to impact nutrition outcomes, particularly given that farming is the main livelihood for a majority of people in India while the country continues to host a large undernourished population. The M.S. Swaminathan Research Foundation (MSSRF) has been promoting FSN both in its regular programmes and Leveraging Agriculture for Nutrition in South Asia (LANSA) project, supported by UKAid. The Key components include production diversification, animal food access, biofortification and capacity building.

The framework utilizes location-specific FSN models promoting sustainable and healthy diets from locally available food resources (plant and animal). The approach has demonstrated feasibility in addressing nutrition deficiencies in farm families as evidence by higher production and consumption of nutrient-rich foods, improved household dietary diversity, and enhanced understanding and acceptance of nutrition-sensitive agriculture (Girard et al., 2012; Pradhan et al., 2021). The framework has shown measurable improvements in the number of items consumed under each food group, frequency of food consumption, and average per capita intake of nutrient-rich foods.

FAO framework for nutri-sensitive agriculture

The framework for nutri-sensitive agriculture, outlined by FAO (Herforth and Ballard, 2016) presents a simplified impact pathway understanding how agricultural interventions influence nutrition by addressing the underlying determinants (food access and care practices) of nutritional status and the broader food and health environment. Six primary outcome areas are identified as food access (through production and market diversity), on-farm availability and diversity, income and livelihoods, women's empowerment, nutrition and food safety knowledge, and natural resource management alongside health and sanitation. Improvements in agricultural production, income, and empowerment drive access to diverse, safe, and nutritious foods, enhance dietary diversity, and support overall nutritional improvements, while effective natural resource management further reduces disease burden. The framework recommends selecting context-appropriate indicators, such as dietary diversity scores (Minimum Dietary Diversity for Women [MDD-W], Household Dietary Diversity) [HDDS], food security indices (Food Insecurity Experience Scale) [FIES], economic measures (income, asset ownership, gender-disaggregated sales), women's empowerment surveys, and health and sanitation access metrics. Projects should tailor evaluation metrics to realistic impact pathways and local context, prioritizing outcomes such as diet quality and food access over direct anthropometric changes, with systematic documentation of broader market, health, and social impacts for holistic assessment. This approach guides the design, monitoring, and evaluation of nutrition-sensitive agriculture, demonstrating how policy, agricultural systems, markets, practices, and resource stewardship together shape improved nutritional outcomes.

IFAD framework

The International Fund for Agricultural Development framework for mainstreaming nutrition-sensitive agriculture is a comprehensive model designed to transform rural agricultural investments and policies, aiming to build resilient and nutrition-enhancing food systems for smallholders and marginalized groups. The framework is guided by principles that prioritize holistic impact byembedding nutrition goals throughout project stages, multisectoral convergence (linking agriculture with health, sanitation, education, and social protection), and equity, with an emphasis on gender sensitivity, empowerment, and support for the most vulnerable. Strategic action areas include; systematic mainstreaming of nutrition into all investment operations and strategies; strengthening

the capacity of stakeholders through training and technical assistance; policy influence and advocacy for enabling environments aligning projects with global and national nutrition strategies; knowledge management and evidence generation using research, monitoring, and evaluation; building organizational capacity for nutrition mainstreaming with dedicated resources.

IFAD's practical project design involves starting with situation analysis, supporting nutrition-sensitive value chains, promoting dietary diversity, empowering excluded groups, advancing nutrition education and behaviour change, and rigorous monitoring with tailored nutrition indicators. Of special note is support for neglected and underutilized species (NUS), which enhance agrobiodiversity and dietary diversity. By integrating nutrition at every level, from policy to implementation, the framework sets benchmarks for global multisectoral nutrition governance, driving the transition to sustainable, inclusive, and nutrition-sensitive agricultural development.

CONCLUSION

Nutri-sensitive agriculture in India is essential for achieving food and nutrition security by integrating diverse, nutrient-rich foods with sustainable production practices. Strengthening these interventions is crucial for environmental sustainability and improved health outcomes for the population. Learning from leading frameworks such as those of the FAO, IFAD, UNICEF, and context-specific models like the Farming Systems for Nutrition (FSN), it is evident that integrating nutrition into agriculture demands a multisectoral and participatory approach, emphasizing gender equity, crop diversification, biofortification, and inclusive value chain development. These frameworks highlight the importance of empowering women, promoting dietary diversity, enhancing knowledge, attitudes, and practices (KAP), and strengthening behavioural communication to encourage the adoption of nutritious choices from farm to consumer. By operationalizing these principles, India can transform its agricultural sector into a primary engine for nutrition security and sustainable development, ensuring that improvements in production directly translate into improved nutritional outcomes for all segments of society.

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Enhancing acclimatization of *Coffea arabica* F1 hybrids through poultry manure biochar amendment

S.F. BILLA* AND K.N. KOME-NDOBIDE

Institute of Agricultural Research for Development (IRAD) Foumbot, Cameroon, PO Box 163 Foumbot

**sammybilla98@gmail.com*

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ABSTRACT

Coffea arabica F1 hybrids offer elite agronomic traits (high yield potential and disease resistance), but they remain difficult to propagate at scale due to high mortality during the acclimatization phase. This study investigated the potential of poultry manure biochar (PMB) to improve substrate properties and enhance the survival and physiological vigour of Starmaya mini-cuttings. Using a Randomized Complete Block Design (RCBD), rooted cuttings were grown in substrates amended with PMB at 0%, 2.5%, 5%, and 10% v/v. Survival rate, root and shoot biomass, root-to-shoot ratio (RSR), and chlorophyll content (SPAD) were assessed over 12 weeks, with an extension phase to monitor post-acclimatization resilience. Results indicated that PMB significantly altered substrate pH and nutrient retention. A 5% PMB amendment was identified as the optimal dose, increasing survival rates to 92.1% compared to 68.9% in the control. This treatment also maximized root biomass (0.87 g/plant) and maintained a balanced RSR (0.6). While higher application rates (10%) induced phytotoxicity linked to elevated salinity, the 5% treatment demonstrated sustained benefits without mineral fertilization. These findings suggest that PMB at a modest rate offers a sustainable, waste-derived solution to the critical bottleneck of nursery acclimatization for elite coffee hybrids.

Key words: Circular economy, mini-cuttings, nursery management, seedling vigor, starmaya, substrate amendment, waste valorization

INTRODUCTION

The rapid development of resilient *Coffea arabica* varieties in the 21st century is essential to counter the threats of shifting climate patterns, rising temperatures, and escalating pest and disease pressures (Marie et al., 2020; World Coffee Research, 2024). Recent advancements in genome editing, such as the clustered regularly interspaced short palindromic repeats CRISPR-Cas9 genome engineering technology (CRISPR/Cas) are revolutionizing crop improvement by enabling the development of varieties that are more productive and resilient to these very stresses (Das et al., 2025). With the land suitable for Arabica coffee cultivation projected to decline sharply, enhancing tree productivity is critical to meeting global demand (Marie et al., 2020). To address these urgent challenges, researchers and international organizations are prioritizing F1 hybrids, a high-performing class of varieties designed to ensure long-term crop stability and farmer prosperity (Georget et al., 2019; Bertrand et al., 2021; World Coffee Research, 2024).

While F1 hybrids are relatively a recent introduction to the coffee sector, they are increasingly viewed as a cornerstone of the future of the coffee industry (World Coffee Research, 2024). These varieties are characterized by hybrid vigor, which manifests as higher yields, broader climate adaptability and enhanced resilience against stressors such as frost, coffee leaf rust and drought (Bertrand et al., 2011; Georget et al., 2019).

The economic advantages of adopting high-performing hybrid varieties over traditional cultivars have been consistently demonstrated across various crops; for instance, front-line demonstrations in sweet corn recorded a 238% yield increase by adopting hybrid varieties (Priyadarshini and Nath, 2025).

Additionally, F1 hybrids offer a significant strategic advantage by accelerating the breeding cycle, reducing the timeline from initial development to commercial release to just 10-20 years, compared to the 25–30 years required for traditional pure line varieties (Mc Cook and Montero-Mora, 2024). Looking forward, the integration of precision breeding tools such as CRISPR- Cas9 genome engineering technology with conventional methods offers further potential to enhance these traits rapidly and sustainably (Das et al., 2024).While F1 hybrids offer high performance, they present risks for farmers accustomed to saving seeds, as F1 offspring do not breed true (Georget et al., 2019). Furthermore, large-scale production is constrained by the high cost of elite germplasm production, driven largely by significant losses during the acclimatization phase (Mondal et al., 2023).

The transition of rooted cuttings from a controlled greenhouse to an external (*ex vitro*) environment, known as acclimatization, represents a primary limiting factor in the successful micropropagation of many plant species (Hazarika, 2003; Jagiełło-Kubiec et al., 2021). During this phase, the micropropagated plants with under developed root systems and stomatal control often struggle to survive after transfer because they are accustomed to high humidity, low light, and a sterile, sugar-rich, autotrophic environment, making them vulnerable to environmental stress (Hazarika, 2003; Grossnickle, 2012). Mortality rates during this stage can reach 30-40%, rendering large-scale deployment financially prohibitive for many nurseries (Politud and Avako, 2016). Therefore, a successful acclimatization ensures high survival rates of the rooted mini-cuttings through environmental regulation by gradually reducing humidity and increasing light intensity in the tunnel to stimulate stomatal function, the selection of optimized substrates such as soil-sand-organic mixtures to promote robust root development (Nogueira et al., 2022), and the implementation of in vitro preconditioning such as adjusting sucrose levels or improving container ventilation to physiologically prepare the plantlet for weaning (Pires et al., 2023).

Concurrently, the rapid expansion of poultry farming in tropical regions generates vast quantities of manure. Direct application of raw manure to sensitive nursery substrates often leads to phytotoxicity due to high ammonia content and low carbon-to-nitrogen (C/N) ratios (Kumari et al., 2024). However, thermochemical conversion via slow pyrolysis will produce P-enriched biochar with surface-bound calcium phosphates, available P from <0.1% to 1.5-2.5%, reduced NH_3 loss, and surfaces for lactic acid bacteria (Bruun et al., 2012; Hadroug et al., 2019). Raw biochar produced from crop wastes via an Elsa barrel pyrolyser (300-500 °C) has high porosity and stability but low concentrations of readily available N, P, and K. Its negative surface charge binds cations well but does not supply them initially (Domingues et al., 2017). Loading raw poultry manure into a specialized container (such as the Elsa barrel, a pyrolysis metal container) for slow pyrolysis keeps the process efficient while producing high-quality biochar (Cantrell et al., 2012; Chen et al., 2017; Hadroug et al., 2019). This method allows for the thermal decomposition of manure into a porous carbonaceous material, which enhances nutrient management and increases the Cation Exchange Capacity (CEC) by 20-35% compared to raw poultry manure (Cantrell et al., 2012; Hadroug et al., 2019).

Poultry Manure Biochar (PMB) is characterized by high nutrient density (specifically P and K) and an alkaline pH, making it a potentially ideal bio-fertilizer or recycled organic residual amendment for the acidic substrates typical of tropical coffee nurseries (Dasgan et al., 2023; Côté and Khiari, 2026). However, while the general benefits of biochar are documented, the specific application of PMB to mitigate transplant shock in F1 coffee hybrids remains underexplored. Additionally, the optimal dosages required to avoid toxicity are not well established.

This study investigates the potential of PMB as a strategic substrate amendment to facilitate the bio-acclimatization of rooted *Coffea arabica* mini-cuttings. The study hypothesizes that: (1) An optimal dose of PMB will stabilize substrate pH and enhance nutrient bioavailability without inducing salinity stress; and (2) improvements in the rhizosphere will promote robust root architecture, maximizing the survival rate (SR) of F1 hybrid mini-cuttings.

MATERIALS AND METHODS

Site and plant material

The study was carried out at the IRAD Foumbot multipurpose research station located in Cameroon's Western Highland agroecological zone III (1014 m asl.; 5°51'41" N, 10°34'13" E). Characterized by a humid subtropical equatorial climate and rugged volcanic topography, the region receives an average annual rainfall of 2,400 mm distributed through a distinct bimodal pattern. The year is divided into a lengthy rainy season from mid-March to mid-November and a subsequent dry season, during which northeasterly Harmattan winds introduce dusty air and reduced humidity. Throughout these shifts, the environmental conditions remain consistently tropical, with temperatures fluctuating between 15°C and 32°C. The study utilized uniform six-week-old rooted mini-cuttings of the Starmaya F1 hybrid, which were obtained from a specialized clonal propagation facility.

Production and characterization of poultry manure biochar (PMB)

Poultry manure was pyrolyzed in an Elsar barrel retort under slow pyrolysis conditions at 500°C for 40-50 minutes (Billa et al., 2017). The resulting biochar was ground and sieved (<2 mm). Physicochemical analyses included pH (1:10 w/v), electrical conductivity (EC), cation exchange capacity (CEC), and total macronutrient content (C, N, P and K) using standardized protocols.

Experimental design and treatments

A randomized complete block design (RCBD) with four PMB treatments (0%, 2.5%, 5%, 10% v/v) and four replications was implemented. Each plot contained 25 mini-cuttings (total n = 400). The substrate comprised topsoil and sand (2:1 v/v). To eliminate soil-borne pathogens that could confound mortality results, the topsoil was sterilized by autoclaving at 121°C for 1 hour prior to mixing. The substrate was then amended with PMB at specified concentrations. Treatment details with concentration of PMB indicating T_0,T_1,T_2 and T_3 are presented for better clarity.

Acclimatization of F1 coffee hybrids

Plants were transplanted into polybags (10 ×20cm) containing respective treatment substrates. They were maintained under 70% relative humidity and 50% shade for 4 weeks, gradually acclimatized to 25% shade over 8 weeks. Substrate moisture was manually maintained at ~60% field capacity. No external mineral fertilizers were applied during the initial 12-week trial.

Longitudinal assessment (weeks 13-24)

The second phase evaluated sustained resilience by comparing the optimal 5% PMB dose (T_2) to a control (T_0) and a high-input treatment (T_4). Although the 10% PMB treatment (T_3) exhibited a survival rate of 80.5% at 12 weeks, it was excluded from the longitudinal growth assessment. This exclusion was based on qualitative observations of severe phytotoxicity and root system dysfunction; surviving T_3 plants displayed stunted orthotropic growth, leaf chlorosis, and brittle root systems that lacked the capacity for future nutrient uptake. These symptoms indicated that the high salinity of the substrate would inevitably lead to failure during the extended nursery cycle. Consequently, T_3 was deemed unsuitable for the long-term study, which focused on viable production protocols. Phase two focused on sustained resilience, comparing three specific treatments:

T_0: Control

T_2: Optimal 5% PMB dose

T_4: High - input regime (Optimal PMB + Fertilization): Plants from T_2 receiving liquid NPK (20-10-10) at 2 g/l starting Week 13.

Data collection

At 12 and 24 weeks, survival rate (SR), root dry weight (RDW), shoot dry weight (SDW), and root-to-shoot ratio (RSR) were measured.

Survival rate (SR)

The number of green and turgid cuttings showing signs of viability (whether rooted or not) were counted and used to determine the survival rate (SR) of the cuttings using the following equation

$$SR\ (\%) = \frac{Ns}{Nt} \times 100$$

- Ns = Number of surviving cuttings at the end of the assessment period.
- Nt = Total number of cuttings planted at the start of the experiment.

Shoot dry weight (SDW)* and *root dry weight (RDW)

On the day of harvest, five plants were randomly selected from each treatment and carefully separated into shoot (stem + leaves) and root samples. The roots were gently washed under running water to remove all substrate particles. The shoot and root samples were then placed into labelled paper bags and dried in a forced-air oven at 60°C until a constant weight was achieved. The dried samples were immediately weighed using a precision Sartorius analytical balance (0.0001 g) to obtain the shoot dry weight (SDW) and root dry weight (RDW), respectively.

Root-to-shoot ratio

The root-to-shoot ratio (RSR) indicates biomass partitioning between the root and the shoot and is often used as an indicator of plant vigor and ability to survive transplanting. To ensure statistical validity, the dry weights of the five sub-samples were averaged to represent a single value per plot. These plot means (n = 4 replications per treatment) were then used for the Analysis of Variance (ANOVA) and mean separation tests.

SPAD value

The chlorophyll content was determined by measuring the third pair of fully expanded leaves from the top of the orthotropic branch using a Minolta SPAD-502.

Field readiness (FR)

To assess the suitability of plants for out-planting, field readiness (FR) was determined at 24 weeks. FR was defined as the percentage of plants in each treatment meeting specific morphological criteria: having a lignified stem (brown coloration), at least five pairs of mature leaves, and a root system capable of holding the substrate ball intact without disintegrating upon removal from the polybag.

Data analysis

Quadratic regression models were employed to describe the response curves to PMB dosage; this approach was specifically chosen to test for an optimal dosage threshold beyond which benefits might decline. Data normality was verified, and means were compared via Tukey's HSD ($p < 0.05$) using IBM SPSS Statistics version 20. The data for survival rate (SR), root dry weight (RDW), and shoot dry weight (SDW) were all best fit by a second-order polynomial equation ($y=a + bx + cx^2$). The resulting models and high coefficients of determination (R^2) were summarized below:

Survival Rate (SR): $SR = 68.5 + 9.1x - 0.85\ x^2$ ($R^2 = 0.95$).

Root Dry Weight (RDW): $RDW = 0.63 + 0.15x - 0.012\ x^2$ ($R^2 = 0.91$).

Shoot Dry Weight (SDW): $SDW = 1.17 + 0.20x - 0.019x^2$ ($R^2 = 0.93$).

RESULTS AND DISCUSSION

Physico-chemical properties of the growth substrates

The chemical properties of the base components (soil, sand, and PMB) used to formulate the treatments are presented in Table 1. Based on the volumetric incorporation rates, the calculated substrate pH for the optimal treatment (T_2, 5% PMB) was approximately 6.0, representing a slight liming effect from the alkaline biochar (pH 9.3). This adjusted pH, combined with the elevated CEC, created the physicochemical environment responsible for the significant improvements in nutrient uptake observed in the subsequent growth analysis.

Table 1. Physico-chemical properties of the base substrates (soil, sand, and poultry manure biochar) used in the study

Parameter	Unit	Soil: sand media (2:1 by volume)	Poultry manure	PMB
Pyrolysis temperature	^oC	-	-	500
Yield	%	-	-	45.2
pH (1:10 w/v H_2O)	-	5.9	8.77	9.3
EC	dS m^{-1}	0.34	0.4	2.56
Total carbon (C)	%	22.34	30.17	48.7
Total nitrogen (N)	%	0.43	2.37	2.1
Available phosphorus (P)	g kg^{-1}	0.31	25.3	28.0
Total potassium (K)	%	0.13	3.22	4.1
CEC	cmolc kg^{-1}	20.08	19.74	45.8
Sand	%	50.43	-	-
Silt	%	20.32	-	-
Clay	%	29.25	-	-

PMB: Poultry manure biochar; EC: Electrical Conductivity; dS: deciSiemens; CEC: Cation Exchange Capacity; cmolc: centimoles of positive charge.

Influence of biochar on substrate pH and salinity

Pyrolysis increases pH by concentrating basic cations and forming carbonates, creating a nutrient-dense carbon skeleton essential for neutralizing the acidic soils of the Western Highlands. This process occurs as volatile components like hydrogen and oxygen are lost, leaving behind concentrated mineral nutrients such as phosphorus, potassium, calcium, and magnesium. This chemical transformation makes Poultry Manure Biochar an effective amendment for stabilizing soil acidity and improving nutrient availability in tropical highland environments. The control substrate (pH 5.9) was slightly acidic. PMB addition linearly increased substrate pH, reaching 6.4 at 10% PMB (Fig. 1). The 5% PMB treatment adjusted the substrate to pH 6, the recognized physiological optimum for Arabica coffee nutrient uptake. Electrical Conductivity (EC) increased with PMB but remained below salinity stress thresholds in the 0-5% treatments.

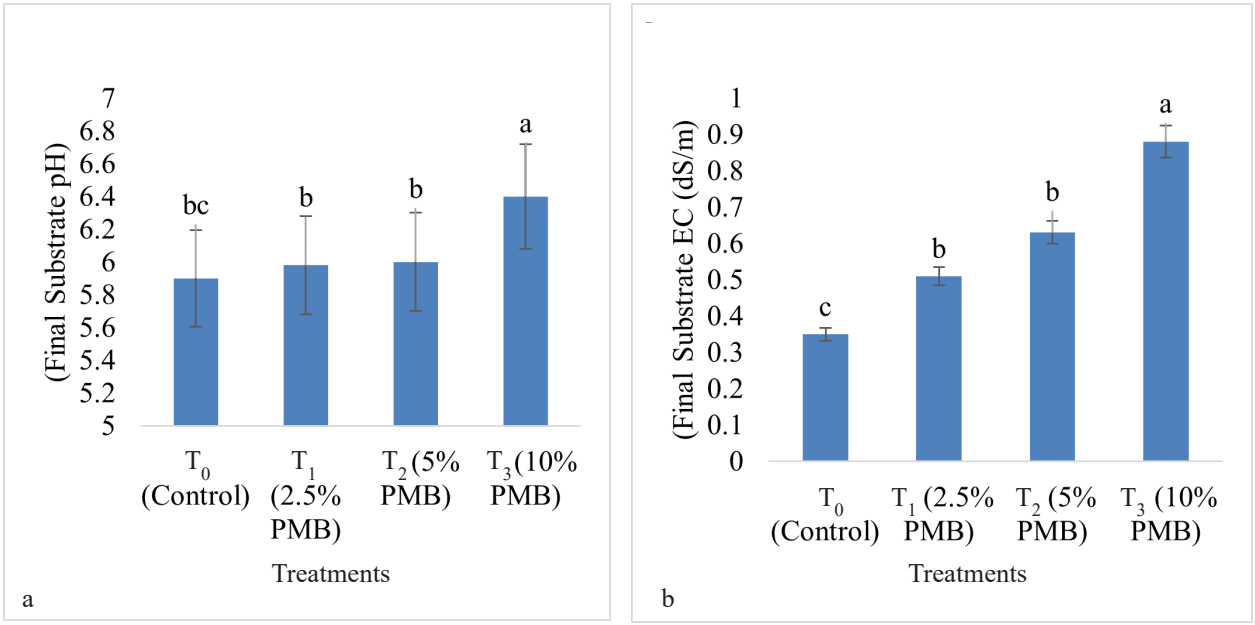

Fig. 1 (a and b). Changes in substrate pH and electrical conductivity (EC) following amendment with varying levels of poultry manure biochar (PMB). Means followed by the same letter in a column are not significantly different ($p<0.05$).

Survival rate, root morphological attributes and acclimatization success

PMB significantly enhanced survival rates (Table 2). The 5% PMB treatment (T_2) achieved the highest survival rate (92.1%), a 34% increase over the control (68.9%). The 10% treatment (T_3) resulted in significantly lower survival (80.5%) and reduced root length, indicating a toxicity threshold at higher PMB concentrations.

Table 2. Influence of poultry manure biochar (PMB) amendment on survival rate and root morphological traits of *Coffea arabica* F1 hybrid mini-cuttings at 12 weeks after transplanting

Treatment	Survival rate (SR %)	Root length (mm)	Number of roots
T_0 (Control)	68.9^c	115^c	7.5^d
T_1 (2.5% PMB)	90.1^b	145^b	10.2^b
T_2 (5% PMB)	92.1^a	159^a	12.8^a
T_3 (10% PMB)	80.5^d	140^b	8.9^c
SEm (±)	1.25	4.30	0.55
CD5%	3.82	13.20	1.69

Note: Means followed by the same letter in a column are not significantly different ($p < 0.05$). SEm **(±)** = Standard Error of Mean; CD5% = Critical Difference at 5% level

Biomass production and allocation patterns

The 5% PMB amendment promoted greater biomass accumulation (Table 3). T_2 yielded a 35% increase in root dry weight (RDW) compared to the control. Furthermore, T_2 achieved an optimal root-to-shoot ratio (RSR) of 0.6, suggesting a balanced allocation of resources to water-absorbing roots versus transpiring shoots.

Table 3. Effect of different levels of poultry manure biochar (PMB) on biomass accumulation and root-to-shoot ratio of *Coffea arabica* F1 hybrid mini-cuttings at 12 weeks after transplanting

Treatment	Root dry weight (RDW, g/plant)	Shoot dry weight (SDW, g/plant)	Root-to-shoot ratio (RSR)
T_0 (Control)	0.64^{c}	1.18^{c}	0.54^{c}
T_1 (2.5% PMB)	0.75^{b}	1.30^{b}	0.58^{b}
T_2 (5% PMB)	0.87^{a}	1.45^{a}	0.60^{a}
T_3 (10% PMB)	0.71^{bc}	1.22^{bc}	0.58^{b}
SEm (±)	0.025	0.041	0.012
CD5%	0.077	0.126	0.037

Note: Means followed by the same letter in a column are not significantly different ($p < 0.05$). SEm (±) = Standard Error of Mean; CD5% = Critical Difference at 5% level

Chlorophyll content and photosynthetic efficiency

The physiological health of the plantlets, as measured by SPAD values in the T_2 treatments (45.2), was significantly higher than the control (38.5), reflecting enhanced nitrogen (N) assimilation mediated by the biochar's CEC (Fig. 2). The lower SPAD values in T_3 (10%) suggest that excessive PMB may inhibit nutrient uptake through osmotic stress.

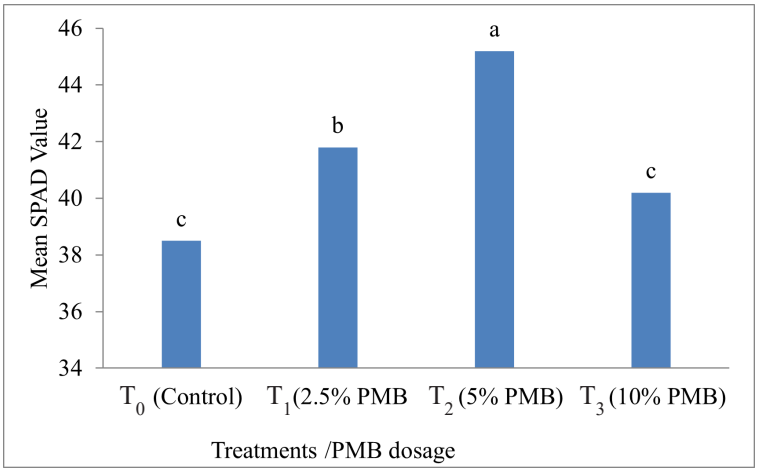

Fig. 2. Effect of poultry manure biochar (PMB) application on chlorophyll content (SPAD values) of *Coffea arabica* F1 hybrid mini-cuttings. Means followed by the same letter are not significantly different ($p < 0.05$). Higher values indicate photosynthetic efficiency and nitrogen assimilation.

Long-term growth resilience and field readiness

By week 24, the survival advantage of the 5% PMB treatment (T_2) persisted (~90.5%), while the control (T_0) experienced a further decline to 65.1% due to nutrient exhaustion (Fig. 3). Although T4 (PMB + fertilizer) treatment achieved the highest total biomass, the T_2 plants exhibited survival rates comparable to T_4 and a balanced RSR, demonstrating that PMB alone can sustain seedling vigor through a standard nursery cycle. The exclusion of the 10% PMB treatment (T_3) from this phase was confirmed as prudent by the observed quality decline in T_3 plants during the interim period; despite initial numerical survival, these plants failed to develop the lignified stems and robust root systems necessary for field readiness (FR).

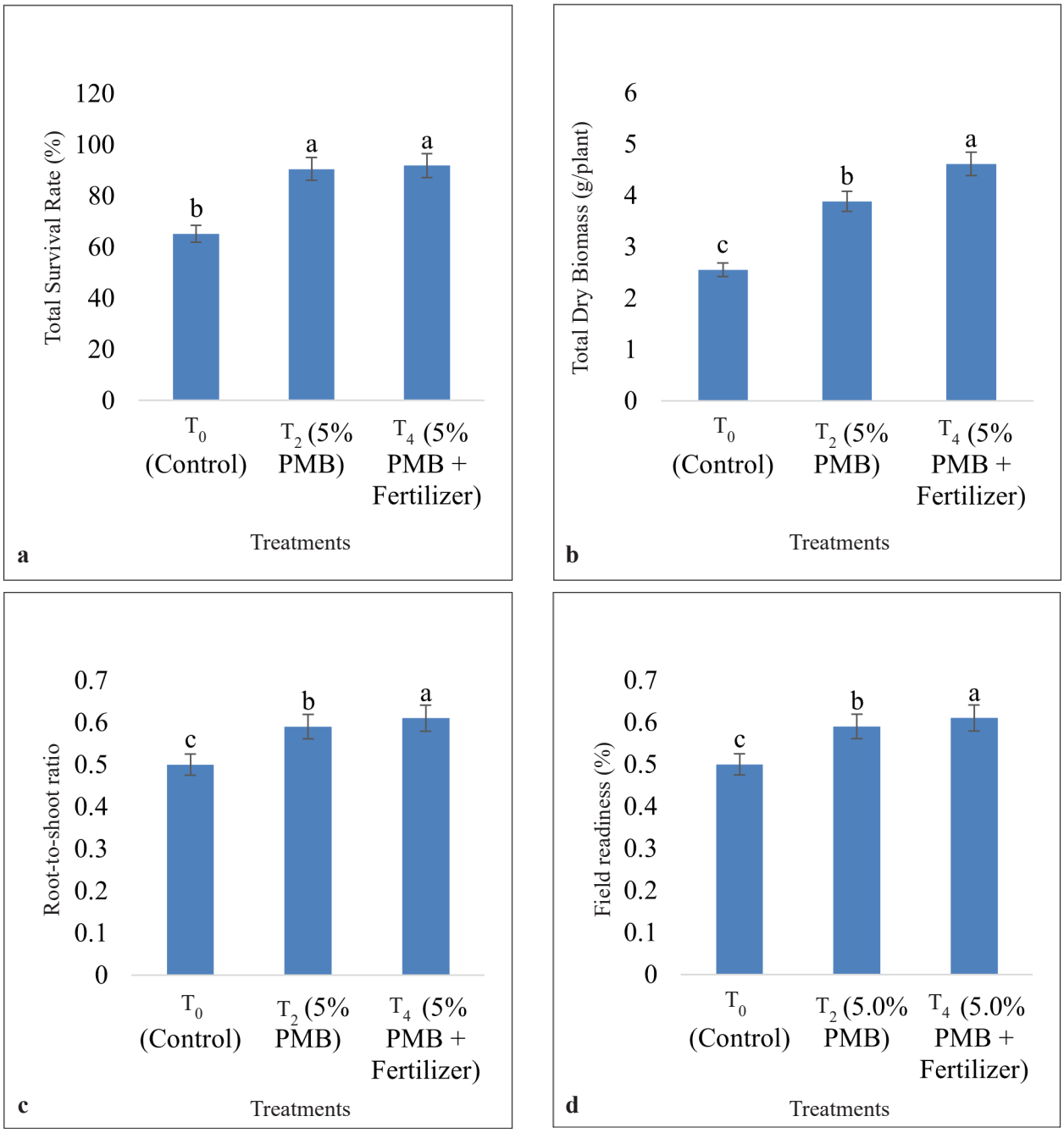

Fig. 3. Performance metrics of *Coffea arabica* F1 hybrid mini-cuttings at 24 weeks: (a) Survival rate (SR, %), (b) Total dry biomass (g/plant), (c) Root-to-shoot ratio, and (d) Field readiness percentage. Means followed by the same letter in a column are not significantly different ($p < 0.05$).

Pearson correlation analysis of growth and physiological interrelationships

To further confirm interrelationship among the key agronomic and physiological variables, a Pearson correlation analysis was conducted (Table 4). This analysis confirmed strong, positive, and significant linear relationships between survival, biomass, and physiological vigour.

Table 4. Pearson's correlation coefficients (r) illustrating relationships among survival, biomass, and physiological parameters in *Coffea arabica* F1 hybrid mini-cuttings

Variable	RDW	SDW	RSR	SPAD
SR (Survival rate)	0.88**	0.90**	0.55*	0.79**
RDW (Root dry weight)		0.92**	0.65**	0.85**
SDW (Shoot dry weight)			0.35	0.88**
RSR (Root-to-shoot ratio)				0.51*
SPAD (Chlorophyll content)				

Note: ** Correlation is significant at the p<0.01 level (2-tailed). * Correlation is significant at the p<0.05 level (2-tailed).

Pearson correlation analysis confirmed a strong positive correlation between RDW and SR ($r = 0.88$, $p < 0.01$) and between RDW and SDW ($r = 0.92$), identifying root development as the primary driver of acclimatization success. The SPAD values showed a highly significant correlation with all three biomass components, indicating that improved physiological health (chlorophyll content) is associated with higher dry matter production.

The findings strongly support the hypothesis that poultry manure biochar (PMB) effectively ameliorates nursery substrates and enhances the bio-acclimatization of arabica coffee mini-cuttings. Historically, raw animal manure has been valued for increasing soil organic carbon (SOC) and cation exchange capacity (CEC) via carboxyl and phenolic group enrichment (Kumari et al., 2024). However, the raw manure management faces logistical constraints, including high moisture and low C/N ratios that complicate composting and transport. Pyrolysis offers a better valorization pathway, destroying pathogens while transforming labile carbon into a stable, long-term sink that maintains nutrient balance (Bruun et al., 2011; Hadroug et al., 2019).

Amendment of substrate properties and mitigation of transplant stress

The performance of Starmaya cuttings in the 5% PMB substrate (T_2) underscores the role of substrate quality in overcoming transition mortality, primarily caused by water stress and nutrient deficiency (Nogueira et al., 2022; Lopez-Merino et al., 2026). PMB addressed these challenges by increasing Water Holding Capacity (WHC) to 65% and decreasing bulk density, which improved aeration essential for aerobic root respiration (Alma and Altikat, 2021; Hadroug et al., 2019).The substrate's high porosity and expansive surface area act as a functional sponge, significantly enhancing water-holding capacity while reducing nutrient leaching (Alma and Altikat, 2021). This physical retention ensures that essential resources remain available within the root zone for extended periods.

Regulation of rhizosphere pH and nutrient dynamics

The acidity (pH 5.1) of the control substrate likely restricted nutrient availability (Table 1). The alkaline nature of the PMB (pH 9.3) served as an effective liming agent, adjusting the T_2 substrate to pH 6.0, which was optimal for the Arabica F1 Hybrid coffee (Hadroug et al., 2019). This stabilization in pH, combined with high P and K content, maximized nutrient uptake, as evidenced by high SPAD values of 45.2 (Nogueira et al., 2022). The high surface area, CEC and porous structure of the biochar enhanced the capacity of the PMB substrate to hold cations and moisture during critical periods of stress (Bruun et al., 2012; Silva et al., 2022). With an annual rainfall averaging 2400 mm, traditional nursery substrates are highly susceptible to nutrient leaching, whereby valuable cations (K^+, Ca^{2+}, Mg^{2+}, and NH_4^+) are washed away before they could be absorbed by the roots. Subsequently, through desorption kinetics, the biochar releases these nutrients back into the soil solution in response to root demand.

This slow-release mechanism effectively explains why the 5% PMB treatment (T_2) sustained vigorous growth for 24 weeks without mineral fertilization, whereas the control (T_0) suffered from nutrient exhaustion. The high nutrient density of the PMB (specifically P and K), coupled with this retention mechanism, creates a consistent nutrient supply that matches the uptake capacity of the developing cuttings. Conversely, the 10% dose (T_3) exceeded the cuttings' tolerance due to high Electrical Conductivity (0.88 dS/m) and excessive pH, leading to salinity-induced growth reduction (Chen et al., 2021).

Effect of biochar on biomass accumulation and partitioning

Superior performance in T_2 was reflected in both above-ground and below-ground biomass. A higher shoot dry weight (SDW) correlates with improved photosynthetic efficiency fueled by PMB-mediated nutrient availability (Evans, 1983). This chemical support translated into significantly higher root dry weight (RDW) and an optimized root-to-shoot ratio (RSR) of 0.60. A balanced RSR is a primary index of nursery stock quality, ensuring absorptive organs can meet the transpirational demands of the shoot (Wilson, 1988). High RDW is the single best predictor of field establishment success, as it enhances hydraulic conductivity and access to water post-transplant (Grossnickle, 2012).

Phytotoxicity thresholds and dose-response relationships

Unlike woody biochars, which are often carbon-rich but nutrient-poor, PMB provides significant amounts of P and K. These findings highlight the importance of dosage precision. The 5% dose successfully buffered the acidic substrate to pH 6.0, optimizing nutrient availability, as evidenced by the SPAD values. However, the decline in performance at 10% PMB serves as a critical warning for nursery managers. The drop in survival and root length at this dose correlates with elevated EC and pH, leading to osmotic stress. Furthermore, the significant rise in substrate pH at the 10% dosage likely induced micronutrient lock-up, particularly limiting the bioavailability of essential cations such as iron (Fe), manganese (Mn), and zinc (Zn). This alkalinity-induced nutrient deficiency, combined with osmotic stress, explains the phytotoxic symptoms observed at higher concentrations. This defines the narrow window between benefit and toxicity for nutrient-dense biochars.

Modulation of root architecture and rhizosphere synergy

The profound increase in total root number, surface area (RSA), and length (TRL) in T_2 (the 5% dosage) provides direct evidence of successful bio-acclimatization. Biochar's porous structure provides nucleation sites for microbial activity and physical channels for root initiation (Mafrica et al., 2025). By physically reducing substrate impedance and improving aeration, PMB essentially primed the Starmaya cuttings for self-sufficiency, resulting in a 92.1% survival rate. The successful acclimatization of Starmaya hybrids depends on the correlation between root dry weight (RDW) and phosphorus (P) uptake. Applying 5.0% poultry manure biochar (PMB) significantly increases RDW, expanding the surface area for intercepting immobile phosphorus ions. This morphological shift could be a direct response to high P availability in the PMB which could have improved the allocation of biomass and physiologically primed the coffee seedlings for growth. Following the dynamics described by Xu et al., (2026), the availability of macronutrients such as P facilitates development of photosynthetic efficiency, a synergy vital for drought tolerance. According to Pang et al., (2010), morphological variations in perennials respond directly to P supply. By maximizing RDW and chlorophyll content, seedlings maintain nutrient flow and energy production during the dry (Harmattan) season ensuring that the plantlets are morphologically primed for the volcanic soils of the Cameroon Highlands.

Economic viability and environmental sustainability of biochar application

For resource-limited nurseries, the dependency on mineral fertilizers can be a significant constraint (Dasgan et al., 2023; Côté and Khiari, 2026). Analogous to the significant

income improvements observed in other sectors through the adoption of improved varieties, such as sweet corn (Priyadarshini and Nath, 2024), the finding that the 5% PMB treatment (T_2) sustained growth and survival compared to the fertilized treatment (T_4) for 24 weeks suggests the potential for PMB to replace or significantly delay the need for mineral fertilization in the early nursery stages. While a detailed cost-benefit analysis is required to quantify specific savings, the valorization of poultry waste (PMB application) into a growth substrate offers a promising strategy for improving resource efficiency. Environmentally, this approach contributes to a circular economy, mitigating the environmental burden of manure management (Bruun et al., 2011).

CONCLUSION

This study confirms that poultry manure biochar at 5% v/v (T_2) is a sustainable soil amendment that significantly improves cutting survival, root development, as well as the physiological health of mini-cuttings of Arabica coffee F1 hybrids during acclimatization. The high pH and cation exchange capacity (CEC) of PMB stabilized the substrate at an ideal pH of 6.0, contributing to a 35% increase in root dry weight and a balanced root-to-shoot ratio (0.6). Post-acclimatization over 24 weeks proved these benefits could be sustained, with T_2 maintaining a 90.5% survival rate compared to the unamended controls T_1 (65.1%). While the supplemental fertilization (T_4) maximized biomass, the PMB-only treatment (T_2) produced high-quality, field-ready plants, offering a cost-effective solution for resource-limited nurseries. Ultimately, valorizing poultry waste into biochar creates a circular-economy tool that optimizes the structural and nutritional stability of the rhizosphere thereby mitigating transplant shock, while accelerating the deployment of climate-resilient coffee clones as well as securing the long-term viability of the global coffee value chain.This study suggests the adoption of this specific PMB dose as a standard protocol for nurseries aiming to scale up the production of elite F1 coffee clones.

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Principal component analysis of mutagen-induced variability and trait interrelationships in greengram (*Vigna radiata* L.)

T.R. DAS[1*] AND B. BAISAKH[2]

[1*]*Division of Genetics, ICAR- Indian Agricultural Research Institute, New Delhi-110012, India*

[2]*Department of Plant Breeding and Genetics, OUAT, Bhubaneswar, Odisha-751 003, India*

[*]*trdas.iari@gmail.com*

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ABSTRACT

The present investigation was undertaken to evaluate the pattern of variability and trait interrelationships among nine quantitative characters of greengram crop in a set of fifteen mutagenic treatments along with the parent variety Sujata as the control using principal component analysis (PCA), which indicated that the first principal component (PC_1) explained 56.18% of the total variation, followed by PC_2 (16.38%) and PC_3 (10.65%), cumulatively accounting for 83.21% of the total variability. Eigenvector analysis revealed that PC_1 was largely influenced by positive contributions from traits such as plant height, pods per plant, seeds per pod, and 100-seed weight whereas days to 50% flowering and days to maturity contributed negatively, reflecting a major contrast between trait groups. The second principal component was dominated by yield per plant, indicating its independent role, while PC_3 was mainly associated with clusters per plant, contributing to secondary variability. The PCA biplot further confirmed the clustering of traits namely plant height, clusters per plant, pods per plant, pod length, seeds per pod and 100-seed weight and the distinct separation of days to 50% flowering and days to maturity, with yield per plant forming an independent axis. Mutagenic treatments, i.e. gamma rays (20 kR, 40 kR) and EMS (0.2%, 0.4%) are closely associated with major contributing traits, appear to be promising candidates for yield improvement whereas MH (0.02%, 0.03%) and 40 kR gamma rays + 0.02% MH were positioned in the opposite direction, showing a negative association with major yield-related traits. Treatments NG (0.010% and 0.015%) and MH (0.01%) were aligned with yield per plant, indicating their specific influence on this trait. The results demonstrate that induced mutagenesis effectively enhanced genetic diversity in greengram and that PCA serves as a reliable tool for identifying key traits and promising genotypes, thereby facilitating selection strategies in greengram breeding programs.

Key words: Genetic variability, greengram, induced mutation, mutagens, principal component analysis, trait association, yield components

INTRODUCTION

Greengram (*Vigna radiata* L.) is an important pulse crop widely cultivated in Asia and other parts of the world due to its high nutritional value and adaptability to diverse environments. It serves as a major source of plant protein and plays a vital role in ensuring food and nutritional security, particularly in developing countries (Nair et al., 2013). Additionally, its ability to fix atmospheric nitrogen enhances soil fertility, making it an integral component of sustainable agriculture systems. Despite its importance, the productivity of greengram remains relatively low due to limited genetic variability and susceptibility to environmental stresses. Yield in greengram is

a complex trait governed by multiple interacting components, which often exhibit varying degrees of association among themselves. This complexity poses challenges for breeders in identifying key traits for selection (Das et al., 2020). The bottlenecks in its improvement have been the lack of variability in different traits and improvement of one trait on its own will affect the performance of other traits because of genotypic correlations between traits (Das and Baisakh, 2019).

Induced mutagenesis has been widely used to create genetic variability in pulse crops. It provides a powerful means of creating new and useful variability in crop plants both in qualitative and quantitative traits (Das and Misra, 2005; Das and Baisakh, 2020). Physical and chemical mutagens induce genes to mutate at rates above spontaneous baselines, thus producing a range of novel traits and broadening the genetic diversity of plants (Das and Baisakh, 2013). Physical or chemical mutagen-induced quantitative variation not only serves as an alternative source of germplasms for natural variation but is also useful in generating appropriately linked gene complexes that are responsible for the improvement in yield and other characteristics of economic interest (Das and Prusti, 2020). Selection of efficient mutagens and their treatment doses is a prerequisite for successful mutagenesis in crop plants as mutagens are potential tools for direct improvement of qualitative and quantitative characters. Several studies have demonstrated the effectiveness of mutagenesis in improving yield and related traits in pulses (Kharkwal and Shu, 2009; Das et al., 2021). However, the evaluation of such variability requires proper statistical tools capable of handling multiple traits simultaneously.

Multivariate statistical techniques, particularly Principal Component Analysis (PCA), have been extensively used in plant breeding to analyze complex datasets. PCA is a particularly useful method for identifying traits that differentiate genotypes. It reduces the dimensionality of data by transforming correlated variables into a set of uncorrelated principal components, each explaining a portion of total variance (Jolliffe & Cadima, 2016). This approach helps in yield associated traits contributing to variability and simplifies the selection process. Correlation analysis complements PCA by revealing the strength and direction of relationships among traits. Positive correlations indicate that traits can be improved simultaneously, whereas negative correlations suggest trade-offs. The interpretation of PCA results involves examining eigenvalues, eigenvectors, and loadings. Eigenvalues indicate the importance of each component, while eigenvectors represent the contribution of individual traits (Abdi and Williams, 2010). Given the importance of multivariate analysis in crop improvement, the present study aims to assess variability among nine traits, evaluate divergence among mutagenic treatments, and identify key traits influencing productivity.

MATERIALS AND METHODS

The experimental material consisted of M_3 genotypes developed from Sujata variety of greengram by fifteen mutagenic treatments i.e three doses each of gamma rays (20, 40 and 60 kR), ethyl methane sulphonate (EMS; 0.2, 0.4 and 0.6%), nitroso guanidine (NG; 0.005, 0.010 and 0.015%) and maleic hydrazide (MH; 0.01, 0.02 and 0.03%) singly, and combined mutagenic treatments of 40 kR gamma rays with 0.4% EMS or 0.01% NG or 0.02% MH. The twelve single mutagenic treatments of gamma rays, EMS, NG, and MH (Sr No. 1 to 12) were coded as G1, G2, G3, E1, E2, E3, N1, N2, N3, M1, M2 and M3, respectively. Three combined treatments of 40 kR gamma rays + 0.4% EMS, 40 kR gamma rays + 0.01% NG and 40 kR gamma rays + 0.02% MH (Sr No.13 to 15) were coded as GE2, GN2 and GM2, respectively. The parent variety (Sujata) used as the control and coded as C (Sr No.16). Observations were recorded on nine quantitative traits i.e. days to 50% flowering (X1), days to maturity (X2), plant height (X3), clusters per plant (X4), pods per plant (X5), pod length (X6), seeds per pod (X7), 100-seed weight (X8) and yield per plant (X9). The data were used to analyze the eigenvalues, percentage variance, cumulative variance and other PCA parameters. PCA was conducted using a correlation matrix. Eigenvectors were extracted to determine trait contributions to principal components. The "R" software (version 3.3.2.) was used for this analysis.

RESULTS AND DISCUSSION

Principal Component Analysis (PCA) was performed to understand the multivariate

relationship among nine plant traits (X1–X9) and to assess the divergence among mutagenic treatments (1–15) in comparison to the control (16). The analysis revealed substantial variability, confirming the effectiveness of mutagenic treatments in generating phenotypic diversity.

Eigenvalues and variance

The PCA results indicated that the first principal component (PC_1) had an eigenvalue of 5.056, explaining 56.18% of the total variation, which clearly dominates the variability structure and the second principal component (PC_2) contributed 16.38% (eigenvalue = 1.474). Together, PC_1 and PC_2 accounted for 72.56% of the total variation, which is sufficiently high to reliably interpret the biplot. Subsequent components contributed progressively less variance: PC_3 (10.65%), PC_4 (7.23%), and remaining components each contributed less than 5% (Table 1). The cumulative variance reached 83.21% up to PC_3 and 90.45% up to PC_4, indicating that the first two to three components capture the major structure of variation. The scree plot showed a sharp decline from PC_1 to PC_2 and gradual flattening thereafter (Fig. 1). The clear "elbow" observed at PC_2 in the scree plot suggests that most of the meaningful variation is captured within the first two principal components, supporting their use for interpretation and graphical representation.

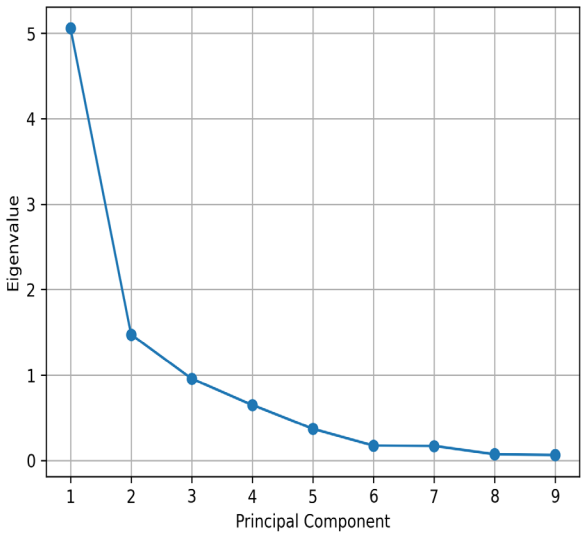

Fig. 1. Scree plot showing eigenvalues of principal components

Trait association and principal component structure

The PCA biplot (Fig. 2) clearly demonstrates that the first principal component (PC_1) is primarily associated with traits X3, X4, X5, X6, X7, and X8, all oriented in the positive direction. The strong clustering and small angular separation among these vectors indicate a high degree of positive correlation. This suggests that these traits are functionally related and may collectively influence yield or productivity. On the contrary, traits X1 and X2 are positioned on the negative side of PC_1, indicating a strong inverse relationship with the above group. This opposition reflects a biological trade-off, where enhancement in X3–X8 may lead to reduction in X1 and X2, or vice versa. Trait X9 is distinctly aligned along PC_2. This indicates that X9 contributes independently to variability and may represent a unique physiological or morphological characteristic. Its vertical alignment indicates that it captures variability not explained by PC_1, highlighting its unique contribution to phenotypic divergence. The predominance of PC_1 in explaining more than half of the total variation highlights the importance of traits X3–X8 in determining overall phenotypic diversity in greengram.

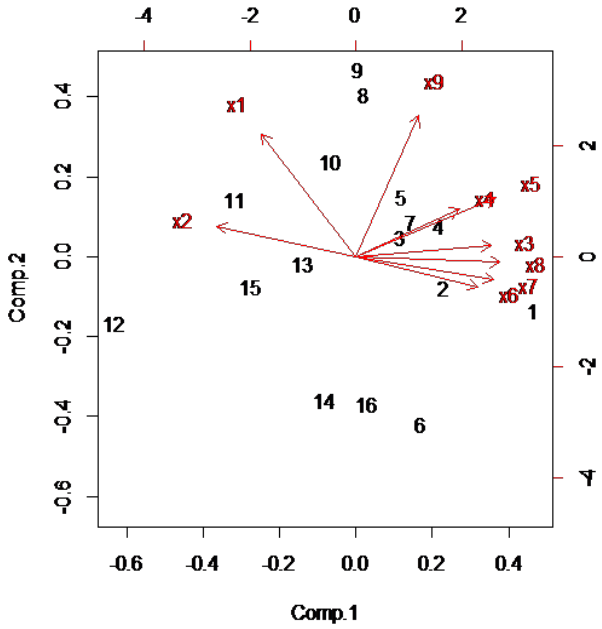

Fig. 2. PCA biplot

Days to 50% flowering (X1), days to maturity (X2), plant height (X3), clusters per plant (X4), pods per plant (X5), pod length (X6), seeds per pod (X7), 100-seed weight (X8) and yield per plant (X9). 1-15 Mutagenic treatments (details in Table 4), 16- control (Sujata)

The PCA biplot (Fig. 2.) clearly demonstrates three major trait groups, i.e., Group I (Positive PC_1 cluster) consists of X3, X4, X5, X6, X7 and X8 traits which are strongly and positively correlated. The alignment of these traits suggests that selection for one trait in this group may simultaneously improve others, Group II (Negative PC_1 cluster) consists X1 and X2 traits are closely associated with each other but negatively correlated with Group I traits, indicating contrasting biological functions or growth patterns and Group III (PC_2 dominant trait) consists X9 trait is relatively independent and contributes distinctly to overall variability, suggesting its importance as a unique selection parameter. Such grouping is typical in mutagenesis studies, where induced variability leads to the emergence of new trait combinations and altered correlations.

Table 1. Eigenvalues and variance

Parameters	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7	PC_8	PC_9
Eigenvalue	5.056	1.474	0.959	0.651	0.372	0.177	0.171	0.074	0.065
% of variance	56.18	16.38	10.65	7.23	4.14	1.97	1.90	0.82	0.73
Cumulative (%)	56.18	72.56	83.21	90.44	94.58	96.55	98.45	99.27	100

The component loading matrix revealed that PC_1 was predominantly influenced by traits X3 (0.810), X4 (0.780), X5 (0.740), X6 (0.690), X7 (0.720), and X8 (0.760), all of which exhibited high positive loadings, indicating their major contribution to total variability (Table 2). In contrast, traits X1 (−0.620) and X2 (−0.680) showed negative loadings on PC_1, suggesting an inverse association with the primary trait group. PC_2 was mainly defined by trait X9, which recorded a high loading (0.850), indicating its independent contribution. The third principal component (PC_3), explaining 10.651% of variance, showed moderate loadings for X1, X2, X6, and X7, representing residual variability.

Table 2. PCA loadings values

Trait	PC_1	PC_2	PC_3
X1	-0.620	0.180	0.310
X2	-0.680	0.120	0.275
X3	0.810	0.050	-0.210
X4	0.780	0.220	-0.145
X5	0.740	0.300	-0.095
X6	0.690	-0.150	0.260
X7	0.720	-0.200	0.185
X8	0.760	-0.080	0.140
X9	0.150	0.850	0.065

The eigenvector matrix (Table 3) revealed that PC_1 was dominated by positive coefficients of X3 (0.368), X5 (0.380), X7 (0.375), and X8 (0.391), while X1 (−0.258) and X2 (−0.377) contributed negatively. This confirms that PC_1 represents a major axis of variation contrasting yield-related traits with early growth traits. PC_2 was primarily influenced by X9 (0.678) and X1 (0.592), indicating a separate dimension of variability. PC_3 showed high contribution from X4 (0.669), suggesting its role in secondary variation.

Table 3. Eigenvector matrix for traits

Trait	PC_1	PC_2	PC_3	PC_4
X1	-0.258	0.592	0.301	0.168
X2	-0.377	0.145	-0.032	0.457
X3	0.368	0.055	0.289	-0.020
X4	0.280	0.228	0.669	-0.250
X5	0.380	0.284	-0.238	-0.101
X6	0.332	-0.144	0.047	0.735
X7	0.375	-0.109	0.163	0.347
X8	0.391	-0.026	-0.382	-0.149
X9	0.170	0.678	-0.383	0.090

Traits with lower absolute value closer to zero influence the clustering less than those with

largest absolute value closer to unity within the first principal component. The strong positive association among these traits suggests that they may be governed by similar genetic factors or physiological pathways, making them suitable targets for simultaneous improvement. The negative association of X1 and X2 with these traits indicates the presence of trade-offs, which is a common phenomenon in crop improvement programs where enhancement of certain traits may adversely affect others. Madhukumar et al. (2023) reported similar negative association for X1 and X2 in greengram. The distinct behavior of trait X9, primarily influencing PC_2, suggests that it represents an independent dimension of variability. This trait may be controlled by different genetic mechanisms and should be considered separately during selection. Similar findings have been reported in other crop studies where PCA effectively identified key traits contributing to variability (Oladosu et al., 2016). The contribution of PC_3, particularly through X4, suggests the presence of additional variability that may help in differentiating genotypes more precisely. The eigenvector analysis further supports these findings by showing that PC_1 is largely defined by traits associated with yield and productivity. The negative contribution of X1 and X2 to PC_1 indicates their contrasting role, possibly related to early growth or structural traits. The strong loading of X9 on PC_2 highlights its independence, suggesting that it is governed by different genetic factors and should be considered separately during selection. Positive and negative loadings indicate correlation patterns between the traits and components, as observed in previous studies (Jakhar and Kumar, 2018).

Distribution of mutagenic treatments

The distribution of mutagenic treatments across the PCA space revealed considerable divergence (Table 4). Treatments G1, G2 , E1, E2, E3, N1, and G3 exhibited high positive PC_1 scores (ranging from 4.031 - 1.012), indicating their strong association with traits X3–X8. Among these, G1 recorded the highest PC_1 score (4.031), suggesting superior performance for these traits. Treatment G3 recorded moderate scores on PC_1 (1.012) and PC_2 (0.229) with a higher score on PC_4 (1.167), indicating a balanced contribution across traits without strong association with any particular trait group. Similarly, N1 showed moderate PC_1 (1.269) and slightly positive PC_2 (0.409) values, suggesting a stable but non-extreme expression of traits. E3 exhibited a relatively high PC_1 score (1.467) but a strongly negative PC_2 value (−1.978), indicating its association with the major trait group (X3–X8) while being negatively related to trait X9. Conversely, treatments M3, M2, GM2, GE2, GN2, and M1 showed negative PC_1 scores (−5.502 to −0.590), reflecting their association with traits X1 and X2. Treatment M3 exhibited the most extreme negative value (−5.502), indicating maximum divergence in this direction. Regarding PC_2, treatments N3 and N2 showed the highest positive scores (2.211 and 1.902, respectively), clearly indicating their strong alignment with trait X9 and its role in trait-specific variation. The treatment GE2 displayed a negative PC_1 score (−1.208) along with a high positive PC_4 value (1.852), suggesting its divergence from the primary trait clusters and contribution to secondary variability. These intermediate and divergent positions indicate that while some treatments strongly influence specific trait groups, others contribute to overall variability and balance among traits. E3, GN2 and the parent (C) recorded negative PC_2 values (−1.978, −1.687, and −1.735, respectively), indicating lower expression of this trait. The control (16) was positioned away from most mutagenic treatments, reflecting limited variability compared to induced genotypes.

In addition to clearly associated treatments, several treatments exhibited intermediate positioning in the PCA biplot. G3 and N1 were located near the center of the plot, indicating moderate and balanced contribution across traits without strong association with any specific trait group. E3, although positioned on the positive side of PC_1, was separated along PC_2, suggesting its association with major traits (X3–X8) but a negative relationship with X9. N3 showed a strong alignment along PC_2, indicating its specific association with trait X9. GE2 was positioned away from the primary trait clusters, reflecting its divergent nature and contribution to overall variability.

Table 4. PCA scores

Sl. No.	Codes	PC_1	PC_2	PC_3	PC_4
1	G1	4.031	-0.639	-0.569	-0.012
2	G2	1.999	-0.369	-1.717	-0.443
3	G3	1.012	0.229	0.571	1.167
4	E1	1.890	0.368	0.768	0.047
5	E2	1.050	0.711	0.679	0.676
6	E3	1.467	-1.978	1.651	-0.850
7	N1	1.269	0.409	0.226	0.175
8	N2	0.199	1.902	-0.203	-1.068
9	N3	0.048	2.211	0.192	-0.331
10	M1	-0.590	1.119	0.462	-0.060
11	M2	-2.764	0.670	-0.188	-0.073
12	M3	-5.502	-0.776	1.080	-0.056
13	GE2	-1.208	-0.083	-0.588	1.852
14	GN2	-0.736	-1.687	-1.286	0.776
15	GM2	-2.405	-0.355	-1.739	-1.098
16	C	0.239	-1.735	0.662	-0.702

The clear separation of treatments in the PCA space demonstrates the effectiveness of mutagenesis in generating genetic diversity. Treatments such as G1, G2, E1 and E3, which are closely associated with major contributing traits, appear to be promising candidates for yield improvement. The intermediate positioning of treatments such as G3 and N1, as indicated by their moderate PC_1 and PC_2 scores, suggests that these genotypes maintain a balanced expression of traits and may serve as stable genetic material in breeding programs. On the other hand, treatments like M2, M3 and GM2, which are positioned in the opposite direction, show negative association with major yield-related traits and may be useful for improving specific traits represented by X1 and X2. Treatments N2, N3, and M1 were aligned with X9 along PC_2, indicating their specific influence on this trait. The distinct separation of the parent (control) from most treatments further confirms that induced mutagenesis has successfully altered the genetic architecture and enhanced variability. The strong negative PC_2 value of E3 indicates its limited association with trait X9 despite its positive contribution to the major trait group, highlighting the complexity of trait interactions. The high PC_2 value observed in N3 confirms its specific contribution to trait X9, indicating that certain treatments may influence individual traits independently rather than contributing to overall performance. The divergence of GE2, reflected by its negative PC_1 and high PC_4 score, suggests its potential role in broadening the genetic base, which is essential for long-term breeding strategies. The alignment of treatments with specific trait vectors in the PCA biplot indicates that mutagenesis has successfully altered trait expression patterns. Treatments positioned along the direction of X3–X8 are associated with improved yield-related traits and can be considered promising for selection. Conversely, treatments located opposite to these vectors may represent undesirable combinations or alternative trait expressions. The independent positioning of the trait yield per plant and its associated treatments suggests that this trait is controlled by different genetic factors and should be considered separately in breeding programs. Overall, the PCA results provide a comprehensive understanding of trait relationships and genotype performance, facilitating more informed selection decisions in greengram breeding programs. The overall distribution of treatments in the PCA space indicates that mutagenesis has successfully generated significant variability, enabling the identification of promising genotypes.

CONCLUSION

The present study demonstrated that Principal Component Analysis is an effective approach for dissecting complex trait relationships and assessing genetic divergence in greengram. The first two principal components accounted for a substantial proportion of total variability, enabling clear interpretation of trait associations and treatment performance. Traits X3–X8 emerged as major contributors to variability and can be targeted for simultaneous improvement. The trait yield per plant should be considered independently, while days to 50% flowering and days to maturity require careful handling due to their negative association with major traits. Mutagenic treatments, particularly

gamma rays (20 and 40kR) and EMS (0.2%, 0.4%), showed promising potential for enhancing desirable traits, whereas the control exhibited limited variability. Treatments NG (0.010% and 0.015%) and MH (0.01%) were aligned with yield per plant, indicating their specific influence on this trait. The findings highlight the usefulness of induced mutagenesis combined with multivariate analysis in broadening the genetic base and accelerating breeding progress for improving productivity.

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Impact of nutritional garden on health and nutritional security of Lodha tribal women in Mayurbhanj district, Odisha, India

J. BHUYAN

Krishi Vigyan Kendra Mayurbhanj-1, Odisha, PIN-757049, India

Jhuni.chutki@gmail.com

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ABSTRACT

The study was carried out among Lodha women aged 18-45 years of age group in Mayurbhanj district of Odisha to know the impact of nutritional garden (NG) on their health and nutritional security. The questionnaire-cum-interview method was applied for data collection. The data was collected before and after establishment of nutritional garden. The collected data were analysed through SPSS and MSTAT-C software. The nutritional garden intervention showed a notable effect on the haemoglobin levels of Lodha tribal women. Prior to its implementation, only 3.67% of the participants were non-anaemic, while 48.33% were mildly anaemic, 45% moderately anaemic, and 3% severely anaemic. Following the introduction of the nutritional garden, the proportion of non-anaemic women increased to 23%. Mild anaemia rose to 65%, whereas moderate anaemia declined to 12%, and no cases of severe anaemia were observed. In addition, various clinical symptoms including Bitot's spots, constipation, joint pain, scurvy, and changes in general appearance, hair, eyes, skin, face, lips, tongue, teeth, gums, and nails showed improvement after the intervention. The mean scores for these symptoms decreased in most cases when compared to pre-intervention values. However, symptoms related to the face showed a slight increase. Overall, the findings indicate a reduction in disease prevalence and clinical symptoms among the participants after the establishment of the nutritional garden, suggesting an improvement in their overall health status.

Key words: Health, Lodha women, nutritional garden, nutritional security

INTRODUCTION

Despite sustainably producing the food grains, about 172.1 million people in India were malnourished (FAO, 2025). But the irony is that recently, India ranked 101st out of 116 nations in the Global Hunger Index (GHI, 2021; IFPRI, 2022; Lal et al., 2022). Food security is all about gaining or fulfilling the 4 dimensions such as availability, accessibility, utilization and stability (Gross et al., 2000; Hahn, 2000; Kundu et al., 2017; Weingärtner, 2009; Lal et al. 2022), which, when coupled with adequate food intake and good health, improves nutritional status. Nutritional security can be defined by the availability, accessibility of diverse, nutritious food (Ruel, 2013). Thus, achieving food and nutritional security primarily depends on agriculture and integrating nutrition into agriculture that pave the way to attain nutritional security with environmental sustainability by providing nutritious and diverse foods. Sahoo et al. (2019) worked on indigenous phyto-therapy of Kandha tribe for primary healthcare in Kandhamal district, Odisha. They added wild and domesticated plants and their parts to treat various diseases such as skin, dental, stomach disorder, rheumatism, jaundice etc. among the Kandha inhabitants with successful nutritional and health security. Mostly, in tribal dominated pockets of India, the nutritional

deficiencies and related health issues have drawn serious attention. Keeping view of this, the authors have been prompted to raise nutritional gardens with plants of nutritional values in the backyards of selected people in tribal dominated pockets of Mayurbhanj district of Odisha.

The Lodha community in India is classified as a particularly vulnerable tribal group (PVTG), predominantly inhabiting the forested border regions of Midnapur (West Bengal), Mayurbhanj (Odisha) and Singhbhumi (Jharkhand). As per the 2011 Census, the Lodha population is to about 9,088 individuals with a substantial concentration in the Mayurbhanj district, particularly in Morada and Suliapada administrative blocks (GOI, 2011). The socio-economic and health conditions of Lodha settlements in Mayurbhanj are characterized by multiple deprivations. These include poor health status, a high prevalence of malnutrition, growth retardation, reduced physical work and elevated rates of morbidity and mortality. The majority of households fall under small and marginal livelihood categories with rice constituting the principal dietary staple food. While rice serves as a major source of caloric intake, it is insufficient in providing essential micronutrients required for maintaining optimal health. Evidence indicates a high rate of micronutrient deficiencies among men and children in the region. Clinical manifestations such as anaemia, night blindness, keratomalacia, spongy bleeding gums, cheilosis, sore throat, angular stomatitis, and scurvey have been widely reported (Bhuyan, 2021). Such conditions reflect chronic dietary inadequacies, including insufficient intake of protein, energy and critical micronutrients such as iron and vitamin A, which collectively contribute to adverse health outcomes. Despite the recognized importance of vegetables as key sources of micronutrients, their per capita consumption in the Lodha dominated villages remain considerably low. In light of this, the present study aims to assess the effectiveness of nutritional garden interventions in enhancing dietary diversity, health status, and overall nutritional security among Lodha tribal women in the Mayurbhanj district of Odisha.

MATERIALS AND METHODS

The present study was conducted in ten selected villages across the Morada, Suliapada, Baripada, and Shyamakhunta blocks of the Mayurbhanj district, Odisha, considering the higher concentration of Lodha tribal population. The investigation was carried over two year periods i.e from 2019 to 2020. The sample comprised of 300 Lodha women selected through house to house survey. The participants were within the age group of 18-45 years and the pregnant and lactating women were excluded from the study. The survey was conducted for data collection in the selected villages. The enumeration process was conducted systematically, covering respondents from one end to the other to ensure adequate representation and reduce selection bias. Socio-demographic data were collected at the household level using a structured questionnaire that was developed by the author in accordance with the study objectives. Data was collected from the respondents about awareness of nutritional garden, dietary habits, intake of different food groups and occurrence of the diseases within three months through a predesigned questionnaire. A rectangular NG measuring 200 sq. m was established in the backyards of the respondents. The area was further subdivided into eight plots, each measuring 3 m x 4 m, where various vegetable crops were grown. During the kharif season, crops such as brinjal, tomato, cowpea, okra (ladies finger), spinach, cucumber, bitter gourd, and pumpkin were grown. In the rabi season, vegetables including cabbage, cauliflower, tomato, brinjal, koshala saga (*Amaranthus*), green chilli and radish were cultivated. In addition, fruit and perennial plants such as papaya (two nos.), drumstick (two), lemon (one), curry leaf (one), and banana plant (one) were planted. Haemoglobin levels of all the respondents were assessed both before and after the establishment of the NG. Statistical methods such as correlation, regression, and chi-square tests were applied to determine the significance of the results. The different clinical symptoms along with haemoglobin level were analyzed before and after establishment of NG with the help of medical practitioner.

Fig. 1 (a, b, c, d). Group discussion and intervention of nutritional garden at the backyard

RESULTS AND DISCUSSION

The collected data was analyzed and interpreted as follows. It was observed that only one fifth of the respondents (21.33%) were aware about the concept of nutritional garden. But only 7% respondents had knowledge about different types of NG and the vegetable crops grown in the NG and only 6.33% of the Lodha tribal women had knowledge about fruit plants grown in the NG. Though some of the respondents had knowledge about the NG, they were not adopting it properly without any concrete idea about it. The above analysis was found to be statistically highly significant indicating that the Lodha women were not well aware of the concept of nutritional garden (Table 1).

Table 1. Opinion pool from tribal women of sample size 300 over knowledge on nutritional garden

Sl. No.	Parameters	Not aware (Nos.)	Not aware (%)	Aware (Nos.)	Aware (%)	Total
1	Awareness about nutritional garden	236	78.67	64	21.33	300
2	Types of nutritional garden	267	89.00	33	11.00	300
3	Vegetable crops are grown in a nutritional garden	279	93.00	21	7.00	300
4	Fruit plants are grown in a nutritional garden	289	96.33	11	3.67	300
	Chi-square	55.12**		P=0.000		Df=3

Table 2 shown the impact of intervention of the NG over the vegetable intake of tribal women. It was observed that before establishment of the NG, 62% of the Lodha women were adding green leafy vegetables in their daily diet, whereas 67% of them added leafy vegetables to their diet after the intervention of the NG. Similarly, due to establishment of NG 12.7% of the respondents included leafy vegetables in their diet on a weekly basis compared to 11.3% of the respondents earlier. In a similar pattern, 18.7% of the respondents used leafy vegetables in their diet occasionally before the establishment of a NG whereas 19.3% of the respondents cooked leafy vegetables occasionally in their diet after it. Further, it was observed that before the establishment of NG, 8% of the respondents never used leafy vegetables for their cooking whereas it was reduced to 1% after it's establishment.

Table 2. Impact of nutritional garden on vegetable intake of tribal women

Sl. No.	Parameters	Daily (%)	Weekly (%)	Occasionally (%)	Never (%)	Total
1	Vegetables intake, leafy vegetables, before	62.00	11.33	18.67	8	300
2	Vegetables intake, leafy vegetable, after	67.00	12.67	19.33	1	300
3	Vegetables intake, other vegetable, before	59.67	15	12.33	13	300
4	Vegetables intake, other vegetable, after	68.00	16	14	2	300
	Chi square	58.85**	P=0.000			Df=9

The intake of fruits and vegetables before and after the establishment of the NG by the Lodha tribal women is presented in Table 3. The fruit intake by the Lodha tribal women were only 11.13 g per day whereas after the establishment of the NG the fruit intake by them increased to 19.09 g per day which is 71.5% higher. Similarly, the roots and tubers intake by the respondents was 25.44 g per day but it is increased to 50.94 g which is 200% higher after the intervention of the nutritional garden. Before the development of the NG, the leafy vegetables intake by the Lodha tribal women was 24.12 g whereas after the establishment of this, the intake is increased to 71.18 g and increases up to threefold. The other vegetables intake by the respondents was 24.8g per day before the intervention of the NG and the intake was 86.39 g which is increased by 248%. Rahman et al. (2008) agrees with the findings on the NG before and after intervention. They indicated before the demonstration overall per day vegetable production was 318g and after the demonstration, it was 553g. Vegetable consumption was 202g and 364g before and after demonstration per day, respectively. Total calorie uptake from different consumed vegetable was 73 kcal and 111 kcal before and after the demonstration respectively.

Table 3. Impact of nutritional garden on intake of fruits and vegetables

Sl. No.	Food groups	ICMR standard (g)	Mean intake (g) Before	SD	% of ICMR standard	Mean intake (g) After	SD	% of ICMR standard
1	Fruits (g)	100	11.13	1.87	11.13	19.09	2.09	19.09
2	Roots and tubers (g)	100	25.44	6.15	25.44	50.94	9.02	50.94
3	Leafy vegetables(g)	100	24.12	7.39	24.12	71.18	10.18	71.18
4	Other vegetables(g)	100	24.80	6.54	24.80	86.39	9.63	86.39

The fruit, roots and tubers, green leafy vegetables and other vegetables intake before and after the intervention of the nutritional garden are significantly different from each other (P= 0.000) [Table 3]. The above findings on the nutritional garden before and after such intervention corroborates with the findings of Rahman et al. (2008).

Correlations of impact of nutritional garden of paired samples of fruits and vegetables intake have been described in Table 4.

Table 4. Paired samples correlations

Sl. No. of pair	Before and after	N	Correlation	Significance
Pair 1	Fruits, before and fruits, after	300	-0.108	0.061
Pair 2	Roots and tubers, before & roots and tubers, after	300	-0.061	0.288
Pair 3	Green leafy vegetables before & green leafy vegetables after	300	-0.062	0.282
Pair 4	Other vegetables, before & other vegetables, after	300	0.015	0.796

Table 5 indicated that all the vitamins and mineral intake increases significantly after the intervention of the nutritional garden ($p<0.001$). The protein intake by the Lodha tribal woman before the development of the NG was 32.15 g whereas after the development of the NG the protein intake by the respondents was 34.69 g which was increased by 7.9% higher. The carbohydrate intake of the respondents was 173.03 g and 190.13 g before and after the establishment of the NG (t-13.44) and both was more than the ICMR recommendation (130 g). This concluded their diet was rich in carbohydrates both before and after the development of the NG as they were mostly dependent on carbohydrates for their energy requirement.

The mean calcium, iron, vitamin A, thiamine, riboflavin, niacin, vitamin C and folic acid intake by the Lodha tribal women before the introduction of NG were 288.40 mg, 7.50 mg, 349.1 µg, 0.50 mg. 0.47 mg, 4.57 mg, 19.18 mg and 125.52 µg whereas the intake by them after implementation were 297.98 mg [t- (-11.54)], 8.70 mg [t- (-286.32)], 483.63 µg [t- (-4914)], 0.72 mg [t- (-248.99)], 0.77 mg [t- (-337.00)], 6.33 mg [t- (-36.21)], 25.70 mg [t- (-147.25)] and 157.81µg [t- (-3082)] respectively. Comparison with the before and after implementation of NG, implied that the significant difference was found to be very high on the intake of different nutrient and micronutrient like protein, vitamin A, vitamin C, calcium, and iron. Rahaman et al. (2008) found similar findings that homestead vegetable gardening increased the contribution of essential nutrients such as protein, vitamin A, vitamin C, Calcium, and iron in the diet. Vegetable grown in the home garden fulfilled 100% requirements of vitamin C, vitamin A and iron. It also fulfilled 47% of protein and 87% of calcium requirements.

Table 5. Impact of nutritional garden on nutrient intake

Sl. No.	Nutrients	ICMR standard	Mean intake (before)	SD	% of ICMR standard	Mean intake (after)	SD	% of ICMR standard
1	Protein (g)	45.7	32.15	4.16	70.3	34.69	4.49	75.9
2	Carbohydrate (g)	130	173.05	20.26	133.1	190.13	6.99	146.2
3	Calcium (mg)	1000	288.40	5.22	28.8	297.98	10.37	29.7
4	Iron (mg)	29	7.50	1.34	25.8	8.70	0.72	30.0
5	Vitamin A (µg)	840	349.1	4.94	41.5	483.66	25.44	57.5
6	Thiamine (mg)	1.7	0.50	0.04	29.4	0.72	0.09	42.3
7	Riboflavin (mg)	2.4	0.47	0.03	19.5	0.77	0.05	32.0
8	Niacin (mg)	14	4.571	0.26	32.6	7.27	0.65	51.9
9	Vitamin C (mg)	65	19.18	2.02	29.5	26.69	1.31	41.0
10	Folic acid (µg)	220	125.52	2.58	57.05	157.57	27.28	71.6

The intake of carbohydrates, iron, vitamin A, thiamine, riboflavin, vitamin C and folic acid before and after the intervention of NG are highly positively correlated with each other. The rest of the nutrients like protein, calcium and niacin before and after NG intervention are not correlated with each other. But in the paired sample test, the intake of all the nutrients before and after intervention of NG is highly significant at 1 % level of significance. This also concludes that the NG has a positive impact upon the nutrient intake of Lodha women (Table 6).

Table 6. Paired sample t-test statistics impact of nutritional garden on nutrient intake

Paired samples test									
Sl. No. of pair	Before vs after	Paired differences					*t*	Degrees of freedom (df)	Significance (2-tailed)
		Mean	Standard deviation	Std. error mean	95% confidence interval of the difference				
					Lower	Upper			
Pair 1	Protein (before vs after)	-2.53	4.61	0.27	-3.06	-2.01	-9.51	299	.000**
Pair 2	Carbohydrate (before vs after)	17.08	22.02	1.27	14.58	19.58	13.44	299	.000**
Pair 3	Calcium (before vs after)	-9.38	14.08	0.81	-10.98	-7.78	-11.54	299	.000**
Pair 4	Iron (before vs after)	-1.20	0.07	0.00	-1.20	-1.19	-286.32	299	.000**
Pair 5	Vitamin A (before vs after)	-134.53	0.47	0.03	-134.59	-134.48	-4914.00	299	.000**
Pair 6	Thiamine (before vs after)	-0.22	0.02	0.00	-0.22	-0.22	-248.99	299	.000**
Pair 7	Riboflavin (before vs after)	-0.30	0.02	0.00	-0.30	-0.30	-337.00	299	.000**
Pair 8	Niacin (before vs after)	-1.76	0.84	0.05	-1.85	-1.66	-36.21	299	.000**
Pair 9	Vitamin C (before vs after)	-6.52	0.77	0.04	-6.61	-6.44	-147.25	299	.000**
Pair 10	Folic acid (before - after)	-32.30	0.18	0.01	-32.32	-32.28	-3082.00	299	.000**

Impact of nutritional garden on health status

The findings of the study indicated that the introduction of NG had a significant effect on the haemoglobin status of Lodha tribal women. Prior to the establishment of NG, only 3.67% of the respondents were classified as non-anaemic, while 48.33% showed mild anaemia, 45% moderate anaemia and 3% severe anaemia. Following the implementation of NG, a marked improvement was observed: the proportion of non-anaemic women increased to 23%, and those with mild anaemia rose to 65%, whereas the prevalence of moderate anaemia declined to 12%. Notably, no cases of severe anaemia were recorded after the intervention (Fig. 2). These findings are consistent with the results reported by Suri (2020), who documented a reduction in the prevalence of anaemia among women from 91% prior to the establishment of NG to 62% post intervention (Table 7). This concurrence reinforces the potential of NG interventions in improving micronutrient status and reducing anaemia among vulnerable respondents.

The impact of nutritional garden on haemoglobin status of the Lodha tribal women (n= 300) as per WHO classification has been presented in Table 7 (WHO, 2024).

Table 7. The haemoglobin status of the Lodha tribal women as per WHO classification

	Hb level (WHO Standard)	Number of the respondents (n=300)	% of the respondents	Number of the respondents (n=300)	% of the respondents
Category		Before	Before	After	After
Non anaemic	>12.0 gm/dl	11	3.67	69	23
Mild anaemic	10.0-11.9	145	48.33	195	65
Moderate anaemic	7.0-9.9	135	45	36	12
Severe anaemic	<7.0	9	3	-	-

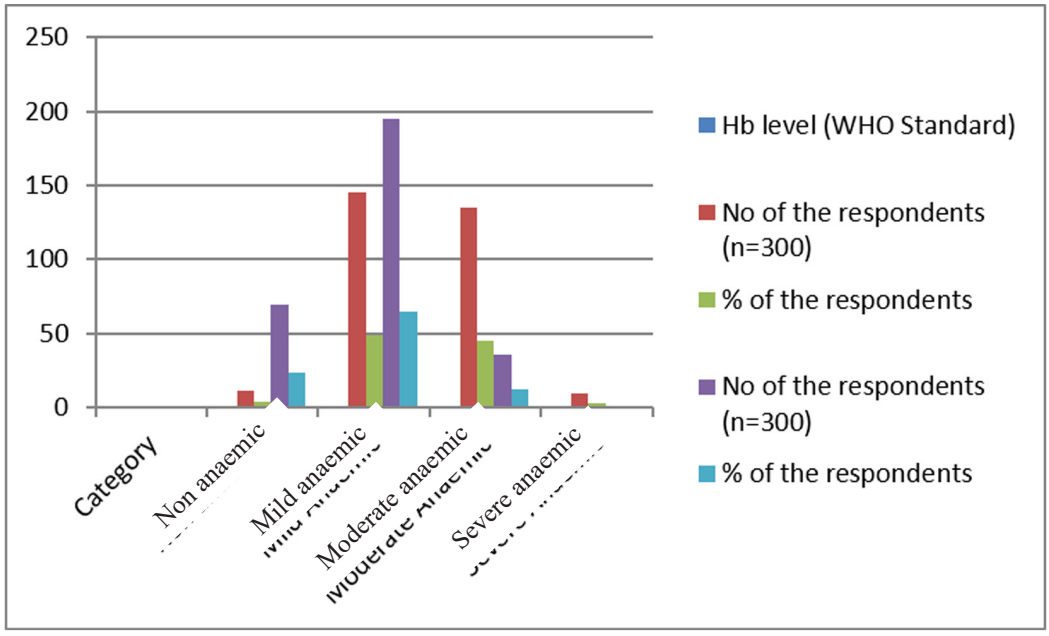

Fig. 2. Impact of nutritional garden on haemoglobin status

Table 8. Impact of nutritional garden on clinical symptoms of Lodha tribal women

Sl. No.	Signs and symptoms	Before frequency (n=300)	Before (%)	After frequency (n=300)	After (%)	Difference
1	General appearance					
	Normal built	21	7	45	15	8
	Thin built	215	71.7	198	66	5.7
	Sickly	64	21.3	57	19	2.3
2	Hair					
	Normal	32	10.7	96	32	21.3
	Dull and dry	205	68.3	168	56	- 12.3
	Easily pluckable	63	21	36	12	- 9
3	Eyes					
	Normal	205	68.3	246	82	13.7
	Night blindness	42	14	24	8	-6
	Pale conjunctiva	53	17.7	30	10	-7.7
4	Skin					
	Normal	45	15	75	25	10
	Xerosis	121	40.3	96	32	-8.3
	Dyspigmentation	134	44.7	129	43	-1.7
5	Face					
	Normal	255	85	276	92	7
	Malar and supra orbital pigmentation	45	15	24	8	-7
6	Lips					
	Normal	45	15	105	35	20
	lesions	125	41.7	96	32	-9.7
	Angular stomatitis	85	28.3	60	20	-8.3
	cheilosis	45	15	39	13	-2
7	Tongue					
	Normal	278	92.7	285	95	2.3
	Fissured	9	3	6	2	-1
	Scarlet and raw	13	4.3	9	3	-1.3
8	Teeth					
	Normal	112	37.3	147	49	11.7
	Carries	98	32.7	75	25	-7.7
	Mottled enamel	90	30	78	26	-4
9	Gums					
	Normal	41	13.7	78	26	12.3
	Spongy and bleeding	259	86.3	222	74	-12.3
10	Nails					
	Normal	45	15	75	25	10
	Brittle	215	71.7	162	54	-17.7
	koilonychia	40	13.3	63	21	7.7

The establishment of the NG was also associated with observable changes in the clinical profile of the respondents, particularly in terms of general body build. Prior to the intervention, only 7% of the women were categorized as having normal body build; this proportion increased to 15% following the implementation of NG. In contrast, the prevalence of thin body build showed a slight decline, decreasing from 71.7% before the intervention to 68% afterward. Similarly, the proportion of respondents classified as having a sickly body build decreased from 21.3% to 19% in the post-intervention phase. These changes suggest a modest but positive improvement in the overall physical condition of Lodha tribal women, indicating that the introduction of NG contributed to better nutritional status and general health outcomes (Table 8).

The NG resulted in significant improvements in clinical indicators related to hair, eye and skin among Lodha tribal women. Prior to the intervention, only 10.7% of participants showed normal hair, which increased substantially to 32% following NG implementation. Conversely, the prevalence of dull and dry hair decreased from 68.3% to 56%, while the proportion of individuals with easy fall hair declined from 21% to 12%. Ocular health indicators also demonstrated notable enhancement. The proportions of respondents with normal eye condition increased from 68.3% before the intervention to 82% afterward. In contrast, the prevalence of night blindness and pale conjunctiva showed a reduction from 14% to 8% and from 17.7% to 10% respectively. Similarly, improvements were observed in skin health, with the percentage of respondents showing normal skin rising from 15% prior to the intervention to 25% following the establishment of NG.

The implementation of NG also had a positive impact on facial clinical symptoms among the respondents. The proportion of women with a normal facial appearance increased from 85% prior to the intervention to 92% afterward. In contrast, the prevalence of malar and supra-orbital pigmentation decreased from 15% to 8% following its introduction. Notable improvements were also observed in lip health, initially only 15% of the respondents showed normal lips whereas it raised to 35% after the establishment of NG. Furthermore, the occurrence of lip related clinical conditions showed a declining trend. The prevalence of lip lesions decreased from 41.7% to 32%, angular stomatitis from 28.3% to 20% and cheilosis from 15% to 13% following the intervention.

There was a noticeable improvement in the clinical symptoms related to the face after the introduction of the NG. Initially, 85% of the respondents had a normal facial appearance, which increase to 92% following its intervention. Prior to the establishment of the NG, 15% of the tribal women showed Malar and supra orbital pigmentation; however, this proportion declined to 8% after the intervention. Similarly, the condition of the lips showed positive changes. Before the implementation of the NG, only 15% of the respondents had normal lips, but this percentage rose to 34% after its occurrence. Clinical signs associated with nutritional deficiencies, including lip lesions, angular stomatitis and cheilosis, were observed in 41.7%, 28.3% and 15% of the respondents, respectively, before the establishment of NG but these were reduced to 32%, 20% and 13% respectively after its introduction.

The research findings revealed that the majority of the respondents had a normal tongue condition. Before the establishment of the NG 92.7% of the women showed normal tongue, and this proportion increased to 95% after the intervention. Nutritional deficiency symptoms affecting the tongue, including fissured tongue and scarlet raw tongue, were observed in 3% and 4.3% of the Lodha tribal women, respectively, prior to the development of NG. Following the introduction of

the NG, the prevalence of these conditions declined to 2% and 3% respectively.

Prior to the study, only 37.3% of the Lodha tribal women had normal teeth, but this proportion increased to 49% after the introduction of the NG. Dental problems such as caries and mottled enamel were reported among 32.7% and 30% of the respondents, respectively, before the establishment of NG. Following the intervention, the prevalence of these conditions decreased to 25% and 26%, respectively.

Before the introduction of the NG, only 13.7% of the respondents had healthy gums. After the establishment of NG, the proportion of the respondents with normal gums increased to 26%. Symptoms related to poor gum health, such as spongy and bleeding gums, were observed in 86.3% of the women prior to the intervention; however, this percentage declined to 74% following the development of the NG.

The findings further indicated improvements in nail health after the introduction of the NG. Before the development of NG, only 15% of the Lodha tribal women had normal nails, whereas this proportion increased to 25% after the intervention. Clinical signs associated with nail abnormalities, such as brittle nails and koilonychias, were observed in 71.7% and 13.3% of the respondents, respectively, prior to the establishment of NG. Following the development of the NG, the prevalence of brittle nails declined to 54%, while Koilonychia was reported among 21% of the respondents (Table 8).

Table 9. Impact of nutritional garden on occurrence of diseases like Bitot's spot, constipation, joint pain and scurvey of Lodha tribal women

Diseases	No of the respondents (n=300) (Before)	% of the respondents (Before)	No of the respondents (n= 300) (After)	% of the respondents (After)	Difference (%)
Bitot's spot	78	26	45	15	11
Constipation	114	38	48	16	22
Joint pain	69	23	57	19	4
Scurvy	81	27	63	21	6

The study demonstrated the effect of the NG on the prevalence of certain health conditions among Lodha tribal women. Initially, 26%, 38%, 23%, and 27% of the respondents were affected by Bitot's spots, constipation, joint pain, and scurvy, respectively. Following the establishment of nutritional gardens in their backyards, these proportions declined to 15%, 16%, 19%, and 21%. This reduction, as illustrated in the graph, indicates an improvement in health status after the intervention (Table 9).

Table 10. Impact of nutritional garden on occurrence of diseases of Lodha tribal women

Paired samples statistics					
		Mean	N	Std. deviation	Std. error Mean
Pair 1	Bitot's spot before	0.2900	300	0.45452	0.02624
	Bitot's spot after	0.1800	300	0.38483	0.02222
Pair 2	Constipation before	0.3633	300	0.48176	0.02781
	Constipation after	0.1433	300	0.35100	0.02026
Pair 3	Joint pain before	0.2300	300	0.42154	0.02434
	Joint pain after	0.1900	300	0.39296	0.02269
Pair 4	Scurvy before	0.2700	300	0.44470	0.02567
	Scurvy after	0.2100	300	0.40799	0.02356
Pair 5	General appearance before	2.1433	300	0.51349	0.02965
	General appearance after	2.0400	300	0.58269	0.03364
Pair 6	Hair before	2.1033	300	0.55409	0.03199
	Hair after	1.8000	300	0.63351	0.03658
Pair 7	Eyes before	1.4933	300	0.77801	0.04492
	Eyes after	1.2800	300	0.63478	0.03665
Pair 8	Skin before	2.2967	300	0.71439	0.04125
	Skin after	2.1800	300	0.80608	0.04654
Pair 9	Face before	1.0200	300	0.14023	0.00810
	Face after	1.0800	300	0.27175	0.01569
Pair 10	Lips before	2.2833	300	0.71045	0.04102
	Lips after	2.1100	300	1.03026	0.05948
Pair 11	Tongue before	1.1167	300	0.43630	0.02519
	Tongue after	1.0800	300	0.36612	0.02114
Pair 12	Teeth before	1.9267	300	0.81865	0.04726
	Teeth after	1.7700	300	0.83632	0.04828
Pair 13	Gums before	1.8633	300	0.34407	0.01986
	Gums after	1.7400	300	0.43937	0.02537
Pair 14	Nails before	1.9833	300	0.53292	0.03077
	Nails after	1.9600	300	0.67818	0.03915

The symptoms of Bitot's spot, constipation, joint pain, scurvy, general appearance, hair, eyes, skin, face, lips, tongue, teeth, gums and nails before the intervention of NG were 0.2900±0.45452, 0.3633±0.48176, 0.2300±0.42154, 0.2700±0.44470, 2.1433±0.51349, 2.1033±0.55409, 1.4933±0.77801, 2.2967±0.71439, 1.0200±0.14023, 2.2833±0.71045, 1.1167±0.43630, 1.9267±0.81865, 1.8633±0.34407, 1.9833±0.53292 whereas the symptoms of Bitot's spot, constipation, joint pain, scurvy, general appearance, hair, eyes, skin, face, lips, tongue, teeth, gums, and nails after the development of NG were 0.1800±0.38483, 0.1433±0.35100, 0.1900±0.39296, 0.2100±0.40799, 2.0400±0.58269, 1.8000±0.63351, 1.2800±0.63478, 2.1800±0.80608, 1.0800±0.27175, 2.1100±1.03026, 1.0800±0.36612, 1.7700±0.83632, 1.7400±0.43937 and 1.9600±0.67818. All the disease occurrences and clinical symptoms were reduced

among the respondents except the symptoms related to face after the intervention of the NG. Hence, it is inferred that the morbidity pattern among the respondents were significantly reduced after the establishment of the NG (Table 10).

CONCLUSION

Mayurbhanj is predominantly inhabited by tribal communities, with nearly 58.04% of its population coming under tribal groups. Across India, tribal populations often face social and economic disadvantages, including limited access to education, healthcare and income opportunities, which ultimately influence their nutritional and health conditions. Ensuring the availability and affordability of nutritious food is essential for maintaining good health. With the aim of improving household nutrition, nutrition garden was established and its impact was assessed among Lodha tribal women. The intervention showed encouraging outcomes, particularly in creating a positive perception toward nutritional improvements within their families. These findings may provide useful insights for policymakers in designing effective strategies to enhance the overall health and nutritional status of the general population, especially tribal women.

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Genetic characterization of Binjharpuri cattle in Odisha, India: Implications for breeding stock selection

J. MOHANTY, P.K. RAUT[1], S.K.DASH[*], D.K. KARNA AND C. MISHRA

Dept. of Animal Breeding and Genetics, OUAT, Bhubaneswar, Odisha, India

[1]Odisha Computer Application Centre, Bhubaneswar, Odisha, India

[*]susantdash46@gmail.com

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ABSTRACT

The present study on 938 Binjharpuri cattle, owned by 230 farmers across 20 villages in four blocks of Jajpur district, the native tract, varying in age, sex and location were assessed. Data were collected through personal interactions with farmers to assess the production and reproduction performance. in 375 cattle (24 male and 351 females)of different age groups. Observations indicated that males exhibited numerically higher body weight than female counterparts without significant difference between sex till maturity, but significantly higher body weight of 252.51 was recorded in males against 206.76 in female counterparts at adulthood. Locality did not have significant effect on any of the biometric traits and body weight at all stages of growth. Binjharpuri cows matured at the age of around 29 months (872.46 days) and dropped their first calf at the age of around 40 months (1184.22 days). The calving interval was around 15 months. Overall lactation length was observed as 293.59 days with average daily milk yield of 4.37 kg in these cows. Moderate but higher heritability estimates were observed at 12 months of age than those at adult stage for body weight and all the conformation traits. Positive and moderate to high genetic and phenotypic correlations were observed between body weight and body measurements. Heritability estimates for reproduction parameters viz. age at sexual maturity, age at first calving, calving interval and production trait like lactation length ranged from 0.29±0.10 to 0.41±0.14.

Key words: Breeding, critical, genetic, potential, selection

INTRODUCTION

Individuals having differential ability not only survive but also reproduce successfully in subsequent generations ensuring continued survival of the said population along with natural selection over many generations under evolution have brought sustained improvement in the population and trigger breeds within species. The Binjharpuri cattle breed, registered with the accession number INDIA_CATTLE_1500_BINJHARPURI_03033, derives its name from its significant presence in the Binjharpur block of Jajpur district, Odisha. Evaluation of characteristics, diversity, distribution, differential performance along with present status of animal genetic resources is highly essential for their sustainable utilization, genetic development and conservation (Panigrahi and Dash, 2018). Hence, the present study was conducted to estimate performance and different genetic parameters on economical traits.

MATERIALS AND METHODS

The native tract includes Jajpur and neighboring regions in Kendrapada, Bhadrak, Keonjhar and Dhenkanal districts (Fig.1 & 2). Under hot and humid climatic profile with summer temperature up to 46^0C, winter with an average of 13^0C and annual rainfall of 1500 mm, these animals are adapted and are contributing to the livelihood,

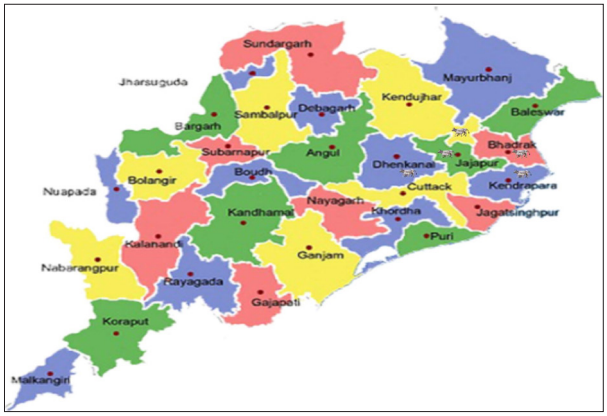

Fig . 1. Native tract of Binjharpuri cattle

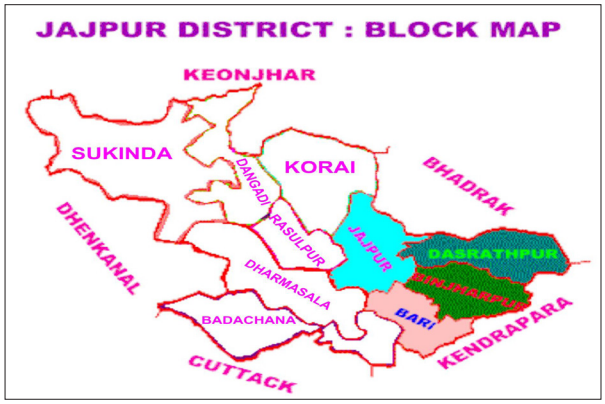

Fig. 2. Block of Jajpur district coloured marks showing high concentration of Binjharpuri cattle

family nutrition, agricultural operations (Fig. 3) and transportation even with promotions for mechanization by Government.

Data were collected through personal interactions with farmers to assess the production and reproduction performance and biometric traits were measured physically for both sexes at various stages of growth.

The body weight at different ages was calculated using Schaeffer's formula i.e.,

Body weight in kg = (L x G^2)/660

Where L = Body length in inches

G = Chest girth in inches.

Length and girth, measured in cm, were transformed to inches for application in Schaeffer's formula (Fig. 18, 19, 20 & 21). A least squares analysis (2-way classification without interaction) was used to examine the effects of sex.

The heritability and genetic correlations were calculated by using the sire component of variance as below:

Estimation of heritability

Heritability: $h^2s = \frac{4\,\sigma^2 s}{\sigma^2 s+\sigma^2 w}$ (Becker, 1975)

Standard error for heritability (h^2) = $\sqrt[4]{\frac{2(1-t)^2[1+(k-1)]^2}{k(k-1)(s-1)}}$

Where, $t = \frac{\sigma_s^2}{\sigma_s^2+\sigma_w^2}$

K = Average number of progeny per sire

Estimation of genetic correlation

Genetic correlation (r_g) between the traits was calculated from sire component of variance in the following formula.

$$r_g = \frac{COV_{S(XY)}}{\sqrt{\sigma^2 s_{(X)} . \sigma^2 s_{(Y)}}}$$

The genetic interpretation is

$$r_g = \frac{COV_A + \frac{1}{4}COV_{AA}}{\sqrt{(V_{A(x)}+\frac{1}{4}V_{AA(x)})\,(V_{A(y)}+\frac{1}{4}V_{AA(y)})}}$$ (Becker, 1975)

Standard Error for genetic correlation r_g:

S.E. for r_g = $\frac{1-r_{g(XY)}^2}{\sqrt{2}}\sqrt{\frac{S.E(h_X^2).S.E(h_Y^2)}{h_X^2.h_Y^2}}$

(Falconer and Mackay, 2009)

Estimation of phenotypic correlation

Phenotypic correlations among different traits were obtained by using the following formula.

$$r_p = \frac{COV_{S(XY)}+COV_{W(XY)}}{\sqrt{(\sigma^2 s_{(X)}+\sigma^2 W_{(X)})(\sigma^2 s_{(Y)}+\sigma^2 W_{(Y)})}}$$ (Becker, 1975)

Error for phenotypic correlation (r_p):

S.E. r_p = $\sqrt{\frac{1-r^2 P(XY)}{n-2}}$ (Goulden, 1962)

Native tract, managemental practices, utility and milk products of Binjharpuri cattle alongwith the process of measurements are presented in Fig. 3 to 21.

Fig. 3. A pair of Binjharpuri bullocks in a ploughing state

Fig. 4. A Binjharpuri bull

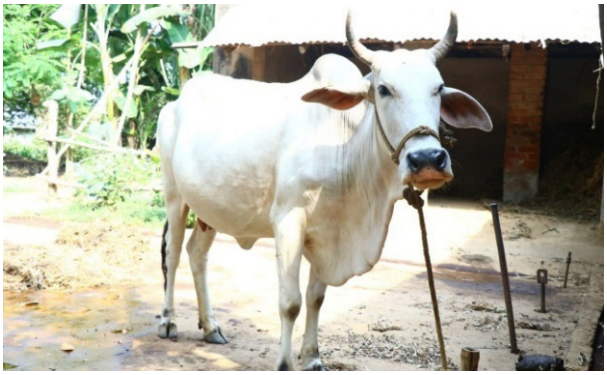

Fig. 5. A Binjharpuri cow

Fig. 6. The Binjharpuri cow dung cake

Fig. 7. A typical shed built for Binjharpuri cattle

Fig. 8a. A Binjharpuri herd

Fig. 8b. Binjharpuri cattle herd at farmer's place

Fig. 8c. Binjharpuri cattle herd under shed after grazing

Fig. 9. A herd of cattle assembled before grazing

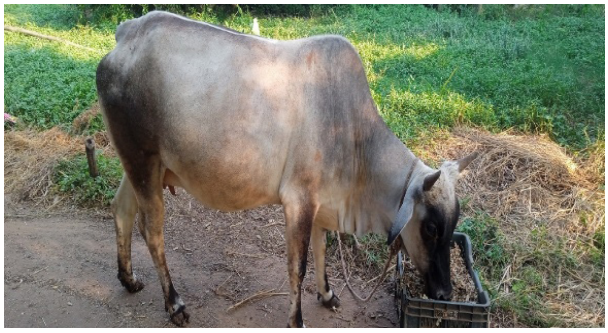

Fig. 10. Binjharpuri cattle provided with groundnut stover

Fig. 11. Cattle provided with Bengal gram chuff

Fig. 12. Binjharpuri cattle provided with rice gruel

Fig. 13. Binjharpuri cattle provided with straw

Fig. 14. Cattle store house for crop residue

Fig. 15. A lady milking Binjharpuri cow

Fig. 16a. Ghee prepared from Binjharpuri cow's milk

Fig. 16b. Curd prepared from Binjharpuri cow's milk

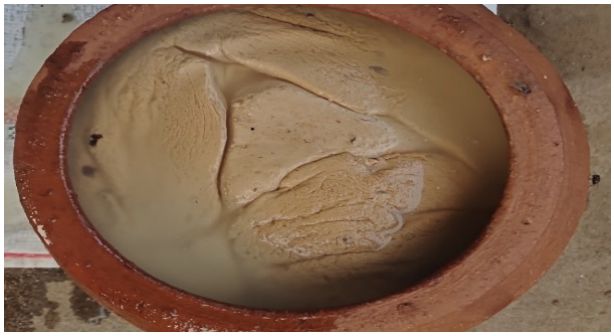

Fig. 16c. Cottage cheese prepared from the cow's curd

Fig. 17a. A black coloured Binjharpuri cow

Fig. 17b. A red coloured Binjharpuri cow with calf

Fig. 18. Measurement of body length

Fig. 19. Measurement of height at withers

Fig. 20. Measurement of heart girth

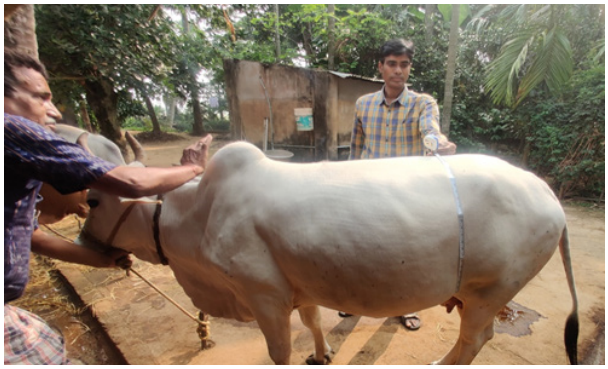

Fig. 21. Measurement of punch girth

RESULTS AND DISCUSSION

Body weight and biometric traits

The body weight and biometric traits of both sexes as presented in Table 1 depict that the least squares overall mean for body weight was 249.58±2.86 kg. The average body weight was estimated as 252.51±5.87 kg in males and 206.76±2.84 kg in females and the height at withers were 117.04±0.87cm for males and 106.18±0.86 cm for females with significant difference between them making males heavier and taller than their female counterparts. Besides, the average body length, heart girth, and punch girth of males were 122.78±0.88, 141.84±1.08 and 147.44±0.78cm and corresponding estimates in females were 107.28±0.80, 132.25±0.78 and 138.65±1.07 cm with significant difference between sexes. Other biometric traits like head, horn, tail, switch, and ear lengths did not show significant difference between male and female Binjharpuri cattle; however, males had numerically higher estimates than female counterparts with respect to ear and switch.

Table 1. Means and standard error for biometric traits of adult Binjharpuri cattle (sex wise)

Traits	Sex		Overall (375)
	Male (24)	Female (351)	
Body weight (kg)	252.51^{a}±5.87	206.76^{b}±2.84	249.58±2.86
Height at withers (cm)	117.04^{a}±0.87	106.18^{b}±0.86	116.35±0.72
Body length (cm)	122.78^{a}±0.88	107.28^{b}±0.80	121.79±0.76
Heart girth (cm)	141.84^{a}±1.08	132.25^{b}±0.78	141.23±0.65
Punch girth (cm)	147.44^{a}±0.78	138.65^{b}±1.07	146.88±0.75
Head length (cm)	43.22±0.25	43.86±0.37	43.68±0.22
Horn length (cm)	12.11±0.18	12.24±0.25	12.21±0.11
Length of tail (cm)	99.12±0.58	97.65±0.68	98.74±0.48
Length of switch (cm)	26.34±0.14	27.32±0.16	27.18±0.10
Length of ear (cm)	22.68±0.18	22.27±0.31	22.32±0.16

*****Means with different superscripts in a row for a trait under sex differ significantly ($p \leq 0.05$)

Lower body weight, height at withers, and heart girth were reported in Binjharpuri cattle by Mahakur et al. (2017); in Kathani cattle by Yadav et al. (2022) and in Badri cattle by Dar et al. (2022) and higher estimates were recorded in Alambadi cattle by Parameswari et al. (2021) and almost similar results were recorded in Binjharpuri cattle by Dash et al. (2013).

Reproduction and production traits

Binjharpuri cows attain sexual maturity at 872.46±1.86 days, give birth to their first calf at an average of 1184.22±1.72 days, and continue to deliver 10 to 11 calves at an average interval of 453.33±1.02 days during their lifespan (Table 2). A lower age at first calving was recorded in Alambadi cattle by Parameswari et al. (2021) and in Kathani cattle by Bhagat et al. (2022). A longer calving interval was observed in Alambadi cattle by Parameswari et al. (2021) and in Kathani cattle by Bhagat et al. (2022).

Table 2. Least square means and standard error for reproduction and production traits Binjharpuri cattle (locality wise)

Sl. No.	Traits	Locality				Overall (423)
		Dasrathpur (112)	Jajpur (98)	Bari (105)	Binjharpur (108)	
1	Age at sexual maturity (days)	868.45±10.24	872.68±8.86	884.45±8.55	864.77±9.26	872.46±1.86
2	Age at first calving (days)	1178.56±12.34	1186.25±9.12	1204.18±9.86	1168.83±11.66	1184.22±1.72
3	Calving interval (days)	452.48±6.86	460.36±7.38	455.54±7.47	445.67±7.55	453.33±1.02
4	Service period (days)	177.24±8.78	184.06±7.22	179.43±7.65	172.54±8.12	178.16±1.15
5	Lactation length (days)	284.66±12.44	290.25±11.37	296.87±12.14	302.68±12.53	293.59±2.24
6	Average daily milk yield (lt)	3.88±0.56	4.22±0.48	4.53±0.45	4.86±0.52	4.37±0.14
7	Lactation yield (lt)	1104.48±86.24	1224.86±78.65	1344.82±82.75	1471.03±78.47	1285.62±10.24
8	Dry period (days)	168.53±10.27	170.19±11.14	159.62±10.27	143.04±9.65	160.19±2.12

The overall least squares mean of lactation length in Binjharpuri cows was recorded at 293.59±2.24 days (Table 2). A shorter lactation length was observed in Belahi cattle by Vohra et al. (2016), in Jhari cattle by Pundir et al. (2020), in Alambadi cattle by Parameswari et al. (2021), in Binjharpuri cattle by Nayak et al. (2022) & Lohith et al. (2022), and in Kathani cattle by Bhagat et al. (2022).

The average daily milk yield among Binjharpuri cows was found to be 4.37±0.14 litter. A lower daily milk yield was reported in Binjharpuri cattle by Nayak et al. (2022) and a higher yield was reported in Belahi cattle by Vohra et al. (2016), in Jhari cattle by Pundir et al. (2020), in Alambadi cattle by Parameswari et al. (2021) and in Kathani cattle by Bhagat et al. (2022).

Genetic parameters

Conformation traits

The heritability estimate for body weight (BW) in adult Binjharpuri cattle was 0.28±0.12. The corresponding heritability estimates for height at withers (HW), body length (BL), heart girth (HG), and pelvic girth (PG) were 0.25±0.11, 0.30±0.17, 0.24±0.11 and 0.26±0.12, respectively (Table 3). Higher heritability estimates for height at withers and body length were reported in Sahiwal cattle by Khan et al. (2018).

Table 3. Heritability, genetic and phenotypic correlations among the biometric traits for adult Binjharpuri cattle

Traits	Body weight	Height at withers	Body length	Heart girth	Punch girth
Body weight	0.28±0.12	0.46±0.23	0.30±0.23	0.22±0.17	0.41±0.23
Height at withers	0.51±0.13	0.25±0.11	0.41±0.22	0.42±0.24	0.38±0.26
Body length	0.38±0.12	0.46±0.09	0.30±0.17	0.34±0.26	0.41±0.24
Heart girth	0.45±0.13	0.36±0.08	0.51±0.06	0.24±0.11	0.25±0.16
Punch girth	0.40±0.09	0.38±0.11	0.33±0.07	0.43±0.09	0.26±0.12

*Diagonal estimates are heritability; above diagonal are genetic correlations and below the diagonal are phenotypic correlations

Reproduction and production traits

The heritability estimate for age at sexual maturity in Binjharpuri cattle was 0.34±0.13. The estimates for age at first calving, calving interval, and lactation length were 0.41±0.14, 0.28±0.11 and 0.29±0.10, respectively (Table 4).

Table 4. Heritability, genetic and phenotypic correlations among different production and reproduction traits of Binjharpuri cattle

Traits	Age at sexual maturity	Age at first calving	Calving interval	Lactation length
Age at sexual maturity	0.34±0.13	0.43±0.05	0.42±0.04	0.35±0.05
Age at first calving	0.78±0.12	0.41±0.14	0.43±0.06	0.24±0.04
Calving interval	0.61±0.07	0.83±0.13	0.28±0.11	0.25±0.05
Lactation length	0.31±0.08	0.32±0.17	0.56±0.19	0.29±0.10

*Diagonal estimates are heritability; above diagonal are genetic correlations and below the diagonal are phenotypic correlations

Higher heritability estimates for age at first calving were reported by Chand (2011) in Tharparkar cows, and Kumar et al. (2016) for Ongole cows. However, Ratan (2018) recorded a lower heritability estimate for age at first calving in Sahiwal cows.

Lower heritability estimates for calving interval were observed by Chand (2011) in Tharparkar cows, Ekka (2012) in Kankraj cows, and Ratan (2018) in Sahiwal cattle. On the other hand, Patro et al. (2005) reported a higher heritability estimate for calving interval in Motu cows of Odisha, than that observed in the present study.

CONCLUSION

The Binjharpuri cows of Odisha are being reared for a small amount of milk. Bullocks are very popular for agricultural operations and carting and for obtaining manure on a low-input basis. The animals were medium in size with an average adult body weight of 260 kg and a height of around 116 cm at withers. The cow attained sexual maturity around the age of 29 months and dropped the first calf around the age of 40 months. The calving interval was approximately 15 months. The cows recorded an average daily milk yield of 4.37 litres with slightly less than a 10-month lactation length. The characteristics of these unique cattle fits to consideration of Binjharpuri as a dual-purpose cattle breed. Recording of moderate heritability of economic traits and moderate to high genetic correlations in the present study. Obtaining dataset on production performance of Binjharpuri cows in the native tract and exploring the results in this study along with individual variations for selection of elite animals under strategic breeding programmes triggering conservation and genetic improvement of this unique cattle breed.

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Economic evaluation of functional chicken meat nuggets formulated with little millet flour

A. DASH[1], B.P. MISHRA[2*], P.K. RATH[3], S.M. SAMANTARAY[1], D. DASH[1]AND J. MISHRA[4]

[1]*Department of Microbiology, College of Basic Science and Humanities, OUAT, Bhubaneswar, Odisha, India*

[2]*Department of Livestock Products Technology, C.V.Sc. & A.H., OUAT, Bhubaneswar, Odisha, India*

[3]*Department of Veterinary Pathology, C.V.Sc. & A.H., OUAT, Bhubaneswar, Odisha, India*

[4] *Krishi Vigyan Kendra, Jharsuguda, OUAT, Bhubaneswar, Odisha, India*

[*]*bidyutpravam@ouat.ac.in*

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ABSTRACT

The need for healthier meat products that are ready to eat or cook has grown as a result of rapid urbanization and lifestyle changes. Although processed meat products provide meat eaters with delicious and convenient options, their high price makes it challenging for the typical consumer to include them into their diet. Thus, the current study's goal is to produce inexpensive and healthier meat products using functional ingredients and compare the manufacturing costs of these products to those of the control product. Nuggets of chicken meat were made using a regulated recipe and extended with a precisely measured amount of little millet flour (LMF). LMF levels were optimized by various trials based on sensory qualities, and those with sensory attributes closer to the control were chosen.To identify the most cost-effective preparation, the price of chicken nuggets after lean meat was substituted with a chosen amount of LMF was examined. The results showed that LMF- incorporated chicken nuggets were less expensive than control items. Thus, it was concluded that the most cost-effective formulation among the levels of incorporation was one that added 6% of LMF at the expense of lean meat.

Key words: Chicken nuggets, cost, economics, functional ingredients, little millet flour

INTRODUCTION

The rise in the cost of various commodities makes it extremely difficult to make animal protein available to all societal segments at a reasonable price. Increasing affluence, urbanization, and the younger generation's increasing understanding of nutrition are the main causes of the rising demand for meat and meat products among consumers. Animal proteins in the form of meat must be incorporated into a daily diet in order to meet the ICMR recommendation for protein consumption of 1 g kg^{-1} body weight/day with Net Protein Utilization (NPU) of 65. Today's rapidly expanding poultry business in India may provide a low-cost supply of proteins with excellent biological value. With 5.18 million tons of poultry meat produced annually, India ranks fourth in the world (BAHS, 2025). Poultry meat has become a mass-consumer commodity worldwide because of its affordable price, high nutritional value, accessibility to all, and lack of associated religious taboos (Sammani et al., 2025; Tramboo and Khan, 2024; Anantaraman, 2022). Chicken meat is chosen over other meats because it has more protein, less overall fat and 50% saturated fat (Mishra et al., 2014) and is therefore chosen over other meats. Meat is regarded as a complete protein since it has all of the required amino acids as well as fatty acids, minerals and vitamins (Rahman et al., 2023). However, meat is devoid of

dietary fibers and also deficient in calcium. Dietary fiber are mostly found in plant materials. Dietary fiber in the diet helps in preventing the occurrence of many lifestyle diseases such as type II diabetes, cardiovascular diseases, obesity, certain cancers and bowel disorders (Kumar et al., 2015). There is growing interest in using extenders, binders and fillers in place of some or all of the meat systems in order to reduce product costs, improve functional qualities and enhance or preserve the expected nutritional and sensory aspects of finished goods for consumers. When added as extenders to meat products, cereals, millets and non-meat proteins enhance yield, texture, functionality and palatability while lowering production costs. This locally accessible cereal flour is far less expensive than other widely used binders/extenders, such as refined wheat flour and maize flour. As a result, it might be utilized to create new, less expensive and functional meat products. The use of LMF as an extender/binder as well as a functional ingredient in emulsion based meat products has not been explored to its maximum. Therefore, in the present study, the production cost of chicken nuggets formulated with optimum level of LMF was determined and compared with control, to conclude and suggest the level of incorporation of LMF for the economic production of functional chicken nuggets.

MATERIALS AND METHODS

Dressed broiler chickens were procured from the local market of Bhubaneswar and manually deboned in the Livestock Products Technology (LPT) Department at OUAT, Bhubaneswar, C.V.Sc. & A.H. Fascia, connective tissue and any separable fat were removed from the lean meat. The meat was then packaged in low density polyethylene (150 gauge) bags and frozen at -18 to -20°C until needed. Food-grade chemicals were obtained from Qualigens, Merck, BDH, and Analar. The refined wheat flour (maida) and LMF were obtained from the local market of Bhubaneswar. To prepare nuggets, condiment and garlic were peeled, cut into small pieces and homogenized in a mixer to obtain a fine paste. Spices were prepared in the laboratory as per a pre-standardized formulation.

Preparation of chicken nuggets

The incorporation level of LMF was optimized by substituting lean meat in the control formulation at three graded concentrations. Evaluation of physicochemical properties and sensory attributes indicated that a 6% inclusion level of LMF was the most suitable. Using this optimized level, functional and extended chicken nuggets were developed by replacing lean meat in the pre-standardized formulation and processing protocol with LMF.

Formulation

The base formulation used for preparing chicken nuggets was standardized as shown in Table 1, and the product developed using this formulation served as the control for comparison with the extended variants. Lean chicken meat was trimmed, cut into small pieces, and minced twice through an 8-mm plate using a meat mincer (Santos, France). The minced meat was then combined with sodium tripolyphosphate (STPP), salt, refined wheat flour, condiments, spice mix, whole egg liquid, and the optimized level of LMF. All these constituents were mixed for about 1 min to obtain a uniform batter or mix. The mix was then placed inside a rectangular mould and kept for steam cooking under pressure for 15 min. After thorough cooking, the product was cooled to ambient temperature, cut into pieces measuring approximately 10 × 15 × 5 mm, and packaged aerobically in LDPE pouches. The packaged nuggets were stored under refrigerated conditions (4 ± 1 °C). A panel of seven trained assessors evaluated the sensory attributes of the functional chicken nuggets at the optimized LMF level using an 8-point descriptive scale (Naveena et al., 2006), where a score of 8 indicated an extremely desirable attribute and a score of 1 indicated an extremely undesirable attribute. The most economically feasible extended nugget formulation was determined by calculating the production cost of the functional and extended chicken nuggets based on the cost of the ingredients and processing procedures involved.

Statistical analysis

The research was carried out in six separate trials (n = 6). SPSS software version 22.0 (Chicago, IL, USA) was used to compile and statistically evaluate the data obtained from all replicates. Using the methods outlined by Snedecor and Cochran (1967), analysis of variance (ANOVA) and Duncan's multiple range test were used to identify significant differences between treatments at a significance level of $p < 0.05$. The control and treatment groups were also compared for each parameter at each sample interval using an independent t-test.

RESULTS AND DISCUSSION

The comparative cost of producing 10 kg of control nuggets and 10 kg of extended or functional chicken nuggets is summarized in Table 2. The calculation includes expenses for all essential raw materials such as deboned chicken meat, refined wheat flour, salt, spice mix, condiments, STPP, refined oil, ice flakes, whole egg liquid, and LMF. The retail prices of these ingredients generally remain stable in local markets; however, bulk procurement from wholesalers or distributors can further reduce the overall production cost.

The total formulation cost for producing 10 kg of the control product was Rs.1988, whereas the cost for the LMF-incorporated formulation was Rs.1868. Thus, both the functional and extended nugget formulations were more economical than the control. The reduced cost for the LMF-based product is largely attributable to the lower price of LMF compared to deboned chicken meat, even when used at the optimized inclusion level.

The overhead expenses associated with producing 10 kg of product are shown in Table 3. These include labour charges (skilled and unskilled), electricity, building rent, packaging materials, water supply, equipment maintenance, loan instalments, LPG usage, and depreciation of equipment at 10% per annum. Overhead cost remained the same for both control and LMF-treated nuggets, amounting to Rs.1740.50 for each 10 kg batch.

Table 1. Basic formulation for preparation of chicken meat nuggets

Items	Quantity (g/100 g of mix)
Chicken meat	70
Refined oil	8
Whole egg liquid	2.75
Refined wheat flour	2.5
Ice flakes	10
Salt	1.5
Condiments	3
Spice mix	2
STPP	0.3
Sodium nitrite (on weight basis)	150 ppm

Table 2. Comparative cost for formulation of 10 Kg control and functional chicken meat nuggets

Ingredients	Rate	Control nugget		6% LMF Treated nugget	
	Rs./ kg	Qty	Rs.	Qty.	Rs.
Chicken (deboned)	240	7	1680	6.4	1536
Refined oil	120	0.8	96	0.8	96
Refined wheat flour	20	0.25	5	0.25	5
Salt	20	0.15	3	0.15	3
Condiments	60	0.3	18	0.3	18
Spices mix	300	0.2	60	0.2	60
Whole egg liquid	120	0.275	120	0.275	120
STPP	200	0.03	6	0.03	6
LMF	40			0.6	24
Total			1988		1868

Table 3. Overhead production cost of 10 kg chicken meat nuggets

Labour charges	Skilled staff = 500 /day Unskilled staff = 450 /day Total= 950/day
Electric charges	30.95 KWh × Rs.6/ KWh = Rs.185.7/ day
Equipment depreciation*	@ 10% per annum i.e. = Rs.26,060 /annum i.e. = Rs.86.86/day (300 working days per annum)
Cost of packaging material	Rs.200/day
Water charges	Rs.20/day
Maintenance cost	Rs.70/day
Rent of building	~ Rs.100/day
Cost of LPG	Rs.50/day
Insurance	Rs.8.33/day
Installment of loan	Rs.69.61/ day
Total overhead cost	Rs.1740.50/ day

Table 4. Total expenditure(Cost/day in Rs.)

Cost analysis	Control	LMF 6%
Raw material cost	1988.00	1868.00
Cost of machineries (Depreciation cost)	86.86	86.86
Cost of electricity	185.70	185.70
Packaging cost	200.00	200.00
Labour cost	950.00	950.00
Rent	100.00	100.00
Maintenance cost	70.00	70.00
Instalment of loan	69.61	69.61
Cost of water	20.00	20.00
Cost of LPG	50.00	50.00
Insurance	8.33	8.33
Total processing expenditure	3728.50	3608.50
Marketing costs (21%)	782.98	757.78
Total	4511.48	4366.28
Gross profit (12%)	541.37	523.95
Cost of 10 kg product	5052.85	4890.23
Cost of 1 kg of product	505.28	489.02
Cost of 100 g packet	50.52	48.90

The total cost of producing 10 kg of chicken nuggets was calculated to be Rs.5052.85 for the control formulation and Rs.4890.23 for the LMF-incorporated product. Accordingly, the cost per kilogram of nuggets amounted to approximately Rs.505.28 for the control and Rs.489.02 for the treatment group. Since both products received comparable sensory ratings, the formulation with the lower production cost was considered more economical. The findings demonstrate that incorporating LMF at the optimized level of 6% reduced the cost of chicken nuggets by Rs.16.26 per kilogram compared with the control formulation (Table 4).

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Gudusia chapra (Hamilton, 1822) [Clupeiformes: Dorosomatidae] in Dhansiri river: A new family record for Nagaland, India

B.R. CHOWDHUARY[1], A.K. TUDU[1], S. RATH[1*], A. GHOSH[2] AND L. KOSYGIN[1]

[1] *Freshwater Fish Section, Zoological Survey of India, Park Street, Kolkata, West Bengal 700016, India*

[2] *Platyhelminthes Section, Zoological Survey of India, New Alipore, Kolkata, West Bengal 700053, India*

[*] *rathshibananda@gmail.com*

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ABSTRACT

Gudusia chapra, the Indian river shad recently placed in the family Dorosomatidae, formerly belonged to Clupeidae, is reported for the first time from the Dhansiri river in Dimapur, Nagaland, representing a new distribution record for the state. This species is widely distributed across Indian riverine as well as brackish water systems and is recognized for its ecological importance and contribution to local fisheries. A lot of morphometric, genomic and molecular studies on *G. chapra* have already been undertaken at different levels including length–weight relationships and heavy metal bio-accumulation studies in various river eco-systems of India and Bangladesh, there has been no prior documentation of its taxonomic account or occurrence in the Dhansiri river, Nagaland.

Key words: Dorosomatidae family, freshwater, *Gudusia chapra*, Nagaland, new record.

INTRODUCTION

The Indian River shad, *Gudusia chapra* (Hamilton, 1822), is a freshwater species belonging to the family Dorosomatidae and placed in the subfamily Alosinae. It was originally described as *Clupanodon chapra* by Hamilton (1822) and its type locality was upper part of Ganges river in Saran, Bihar, India. It was originally described under the genus *Clupanodon* but is currently recognized as a valid species of the genus *Gudusia*, representing an important Clupeid component of riverine systems across the Indian subcontinent (Whitehead, 1985). The distribution of *G. chapra* is primarily concentrated in the Ganges and Brahmaputra river drainages flowing into the Bay of Bengal. Its range also extends to inland freshwater systems of Nepal and Pakistan (Menon, 1999; Shrestha, 1994).

Morphologically, *G. chapra* demonstrates considerable ecological plasticity, adapting to diverse habitat types that include lotic riverine environments as well as lentic lacustrine and ephemeral floodplain systems. This adaptability allows it to inhabit varied freshwater ecosystems, indicating its ecological versatility (Ataur Rahman, 1989). The ecological plasticity of *G. chapra* coupled with its widespread presence underscores its biological importance as a staple resource in artisanal fisheries throughout its native range, where it is a significant bio-resource contributing to local livelihoods and fisheries economies (Jayaram, 2010; Talwar & Jhingran, 1991).

Gudusia chapra is a taxonomically distinct species with ecological and fishery importance in the Indian subcontinent experiencing wide distribution, habitat versatility. Hence, there is a possibility that this species might have been introduced to the Nagaland ecosystem. The well-being of an aquatic ecosystem is assessed by its biodiversity. In aquatic

ecosystems understanding of fish species diversity is complicated due to the existence of several factors and their combinations. Factors like the quality of fish resources, emigration, reproductive potential, physical and chemical characteristics of the aquatic environment are mainly responsible for the diversity and richness of species in aquatic systems (Dash et al., 2018). Moreover, ongoing environmental monitoring underscores the need for regulation to ensure sustainable fishery use and consumer safety in its native habitat.

MATERIALS AND METHODS

The map shows the distribution of *G. chapra* in India including the present study area in Nagaland (Fig. 1). The survey of fish diversity conducted in the Dhansiri river at Thaheku bridge, Dimapur, Nagaland, involved collecting fish specimens using cast-nets, which is a common method for catching a variety of fish species in freshwater environments. The collected specimens were labeled and fixed in 10% formalin for preservation, following the protocol described by Jayaram (2010), which ensures long-term maintenance of morphological features for identification and study. Identification of fish species was based on systematic keys and descriptions provided by Jayaram (2010), well-recognized references in ichthyology; measurements were taken point-to-point with digital callipers on the left side of the specimens and recorded to the nearest 0.1 mm. Counts, measurements, and terminology follow the methodology adopted by Jayaram (2010). Fin rays and scale numbers were counted under a Leica stereo-zoom microscope M205A.

RESULTS AND DISCUSSION

Gudusia chapra is widely distributed across the South East Asia mainly along the river systems of the Indian subcontinent such as the Ganges, the Brahmaputra, the Mahanadi, the Godavari and the Krishna that drain mostly into the Bay of Bengal and few into Arabian Sea. It is commonly found in India in the states like West Bengal, Bihar, Uttar Pradesh, Odisha, Assam and Maharashtra. However, it has

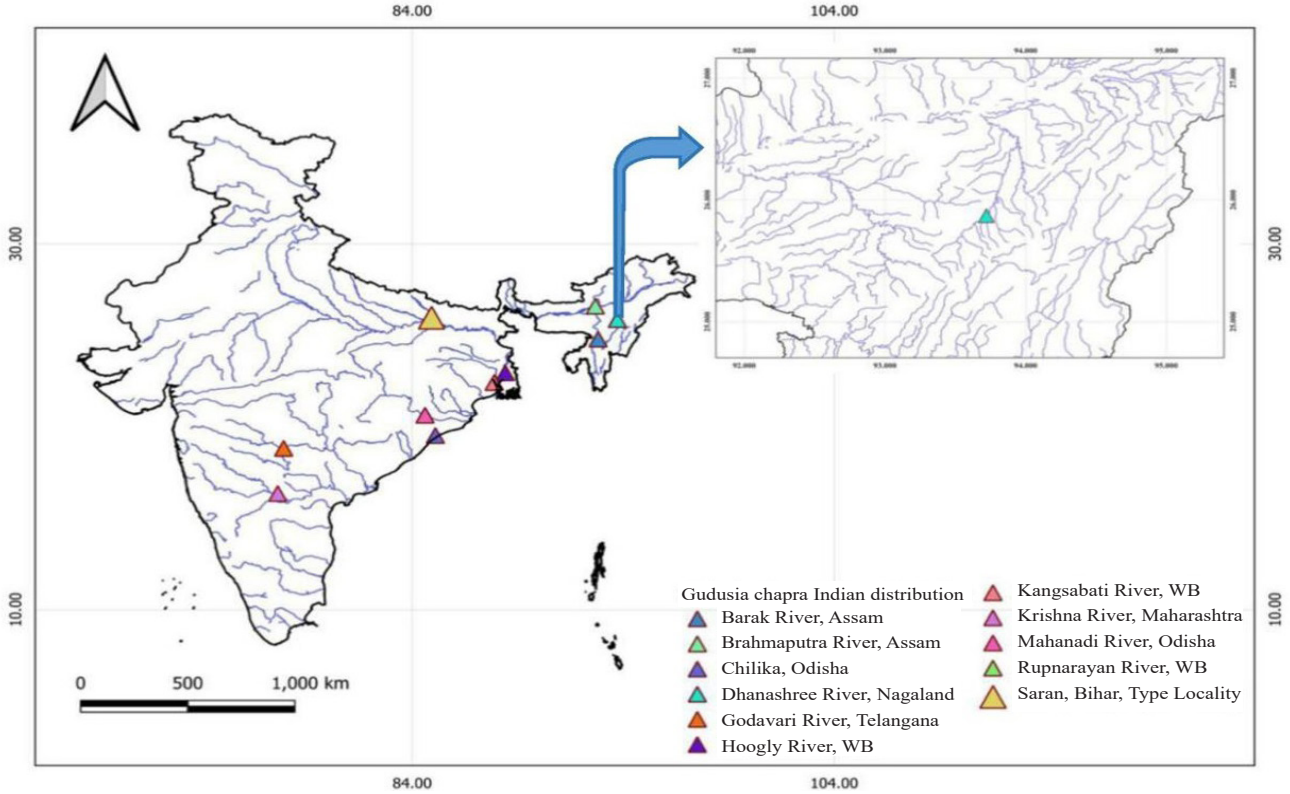

Fig. 1. Map showing distribution of the *Gudusia chapra in* India and present study undertaken in Nagaland

not been reported so far from Nagaland. This species is formally recognised under different scientific synonyms mentioned below.

Clupanodon cagius (Hamilton, 1822). An account of the fishes found in the river Ganges and its branches. Edinburgh & London. i-vii + 1-405, Pls. 1-39.

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Gudusia godanahia (Srivastava, 1968). Fishes of eastern Uttar Pradesh. Vishwavidyalaya Prakashan, Varanasi, India. i-xxii + 1-163

Material examined

ZSI FF 11062, 2 exs., 102.1-108.3 mm SL, India, Nagaland, Dhansiri river, Thaheku bridge Dimapur, 25.86564° N and 93.71722° E, 152 m, 31.01-2025 (Fig. 2).

Distinguishing characters

Gudusia chapra is distinguished from all other species of *Gudusia* by the combination of presence of scutes along the belly; a distinct median notch in the upper jaw. The dorsal fin is positioned approximately equidistant between the snout tip and caudal fin base, with its depressed tip extending behind the vertical from the anal fin origin; single triangular pectoral axillary scale; presence of lateral series. The pelvic fins have seven rays (i7), typically inserted just before the dorsal fin origin, and the anal fin is short and located well behind the dorsal fin base; dark blotch behind gill-opening, often followed by a series of spots along flank.

Fig. 2. A. Lateral view, B. Scutes and C. Shadles of *Gudusia chapra* (Hamilton, 1822), ZSI FF 11062, 108.3 mm SL.

Body is deep, strongly compressed bearing a keel prepelvic scutes 17-19 and post pelvic scutes 11 along the belly; dorsal fin rays iii11-iii12; pectoral fin rays i13; pelvic fin rays i7; with 86 to 89 scales in the lateral line in series; gill rakers are numerous and fine,170 to 180; branchiostegal rays count is six. Additionally, the scale rows are somewhat irregular except along the upper body, and the scales themselves are very small; body with back brown, flanks silvery or golden; blotch behind gill-opening, often followed by a series of spots along flank. The specimen was received from the Platyhelminthes section of ZSI, Kolkata to study the fish parasites. For the purpose, the specimen was dissected. Some tissues from the lateral side were damaged and few scales were also removed. So, the fish specimen although little distorted, the identifying characters were significantly feasible for the studies. The morphometric and meristic measurements have been provided in Table 1. The present specimens match with the general description provided by Talwar & Jhingran (1991). The morphological and meristic characteristics of the two examined specimens corroborate with the earlier studies (Jayaram, 2010; Sani et al., 2010 and Shil et al., 2022), with little variations possibly due to geographic differences.

Table 1. Morphometric characters (in mm) and meristic counts of the *G. chapra* from the Dhansiri river, Thaheku bridge, Dimapur, Nagaland, India. Ranges include values of recorded specimens (N = 2). SD, standard deviation

Morphometry	Ranges	Mean	SD
Standard length (SL) in mm	102.8-108.8	105.8	4.2
In percent of standard length			
Fork length	108.92-111.67	110.29	1.9
Head length	33.55 - 44.36	38.95	7.6
Head depth	30.15-12.74	21.45	12.3
Dorsal fin length	15.37-18.47	16.92	2.2
Pectoral fin length	19.30-20.04	19.67	0.5
Pelvic fin length	10.41-11.31	10.86	0.6
Anal fin length	20.53-23.35	21.94	2.0
Predorsal distance	49.63-51.65	50.64	1.4
Post dorsal distance	38.60-38.81	38.71	0.1
Pre anal distance	71.51-72.08	71.79	0.4
Dist. between pect and pelvic	22.18-24.17	23.18	1.4
Dist. between pelvic and anal	23.54 - 24.17	23.86	0.4
Base of dorsal fin	10.66 -11.19	10.92	0.4
Base of anal fin	17.90 - 19.58	18.74	1.2
Max. body depth	32.17- 32.30	32.23	0.1
Min. body depth	9.56 - 9.63	9.59	0.1
Length of caudal peduncle	9.34-9.56	9.45	0.2
Length of caudal fin	27.85 - 28.31	28.08	0.3
In percent of head length			
Eye diameter	18.86 - 23.84	21.35	3.5
Pre orbital distance	14.47-17.26	15.87	2.0
Inter orbital distance	16.67-22.19	19.43	3.9
Meristic			
Dorsal	iii 11- iii 12		
Pectoral	i 13		
Pelvic	i 7		
Anal	iii 21- iii 22		
Caudal	10 + 9 10 + 9		
Lateral line/series	86-89		
Pre pelvic scutes	17-19		
Post pelvic scutes	11		
Abdominal scutes	28 -30		
Caudal peduncle	30-31		

This species collected from the Dhansiri river at Thaheku bridge, Dimapur, Nagaland, which is the longest and a crucial aquatic habitat of Nagaland supporting rich fish biodiversity. The presence of *G. chapra* in this river underlines the ecological significance of this habitat for sustaining a variety of fish species. *G. chapra* adapts well to these varied habitats and plays an important role in the local aquatic ecosystems in its native South Asian river scape (Sani et al., 2010).

The family-level classification in Clupeidae has undergone various revisions. In this finding is consistent with the higher-level phylogenetic framework of clupeid fishes inferred from mitogenomic data by Lavoué et al. (2013). The data suggests that the lineage I comprising the dorosomatins (*Dorosoma* spp., *Nematalosa* spp., *Clupanodon* spp., *Konosirus* spp., and *Anodontostoma* spp.) along with allied taxa, including members of Alosinae (e.g., *Tenualosa* spp. and *Gudusia* spp.), also members of Pellonulinae, *Harengula* spp., and *Escualosa* spp. was recovered as a strongly supported monophyletic clade. Although these five lineages were not previously delimited based on morphological synapomorphies, such as gill-raker counts, as noted by Nelson (1967, 1970), the mitogenomic evidence provides robust support for their recognition and consolidation within the family Dorosomatidae. *Gudusia chapra* (Hamilton, 1822) and *Gudusia variegata* (Day, 1869) are the two recognized species worldwide mainly present in India and its adjacent countries. Both the species are member of the Dorosomatidae family. A total of 216 species from 29 families were reported in the state of Nagaland (Wewa et al., 2025). *Gudusia chapra* is not included in this checklist, hence being reported for the first time. This species is extensively found in the river systems of India and Bangladesh that flow into the Bay of Bengal. Primarily, *G. chapra* is present in the Ganges and Brahmaputra river basins. *G. chapra* is also present in the same riverine system of Dhansiri in Assam, the neighbouring state. It is also distributed in the Mahanadi river in Odisha and the Krishna river in Maharashtra, the Ganges, Brahmaputra, Godavari and elsewhere in Nepal, Sri Lanka, Myanmar, and Pakistan (Shil et al., 2022; Jhingran 1966). The species inhabits the middle and upper sections of rivers. This species can survive in lentic as well as lotic habitats such as lakes, ponds, ditches, rivers, reservoirs, and inundated fields. It is a commercially important target species for small-scale fisheries in India and adjacent countries (Kumari et al., 2018). The IUCN status of the species is Least Concern. The Dhansiri river, originates from the Laisang peak in the Phek district of Nagaland. It flows northward, draining at Dimapur and Chümoukedima districts of Nagaland, before passing through the Golaghat district of Assam and joining the Brahmaputra at Dhansirimukh. This species already recorded in the Brahmaputra and Barak drainage systems in Assam. During an ichthyological exploration, two specimens an *Gudusia* were collected and deposited at the National Zoological Collections at ZSI, Kolkata, ZSI FF 11062.

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Phytochemical profiling and antibacterial assessment of *Mappia nimmoniana* (J. Graham) Byng & Stull

R.S. DEVI*, S. JETHY AND S. KUMAR

Ambika Prasad Research Foundation, Cuttack, Odisha, India

**rajkumarisupriya01@gmail.com*

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ABSTRACT

Mappia nimmoniana (J.Graham) Byng & Stull, a medium-sized tree, is distributed in the states viz. Odisha, Maharashtra, Goa, Kerala, Assam, Tamil Nadu and Jammu & Kashmir. It is known for its therapeutic potentials including anti-cancer activity. The present study explores the phyto-chemical profiling, antimicrobial activity and DPPH radical scavenging assay of *M. nimmoniana* leaves. The study detected various phyto-chemical compounds such as tannin, saponin, terpenoid, alkaloid, steroid and reducing sugar. Further quantification of the phyto-chemical compounds revealed abundant tannin content (22.15 mg/100 g), phenol content (2.17 mg/100 g), comparatively low flavonoid content (0.458 mg/100 g) and saponin of 5.994 %. Although no significant antibacterial activity was observed against *E. coli*, the aqueous extract showed high radical scavenging activity of 54.04% at the lowest concentration of 0.125 mg ml^{-1}, which correlates with the presence of bioactive compounds in the polar solvents. As the extraction shifts towards non-polar solvents, the radical scavenging activity is reduced to half in the ethanolic extract and is negligible as it reaches the lowest polarity of n-hexane. The present study showed that *M. nimmoniana* could be a source of bioactive compounds for future drug development and also highlighted the need for sustainable extraction of plant parts for drug development works.

Key words: Phytochemical, plant collection, radical scavenging**,** secondary metabolites

INTRODUCTION

Medicinal plants play an important role in India due to their deep-rooted connection with traditional healthcare systems, cultural and religious activities. India is one of the world's richest countries in terms of plant diversity and most people inhabiting in the country still rely on plant-based remedies for primary healthcare. Ayurveda, Unani and Siddha are ancient therapeutic means for treating a wide range of ailments, from common colds to chronic diseases. India has a rich bio-resource of medicinal plants with innumerable bioactive components that are the source of many pharmacological drugs (Jedage et al., 2018). Medicinal plants in India still provide affordable and accessible healthcare, especially in rural and tribal areas, where people have good knowledge of the utilization of medicinal plants. It also contributes significantly to India's pharmaceutical and herbal industries. India is a major exporter of herbal products, raw plant materials and traditional medicines, supporting livelihoods for farmers, collectors and small-scale industries. The growing demand for natural and herbal products has further increased their commercial value. *Mappia nimmoniana* (J. Graham) Byng & Stull is a medium-sized tree with therapeutic importance and distributed in countries like Sri Lanka, China, Taiwan, South East Asia, the Philippines and India (Sharma et al., 2010). In India, it is distributed in Odisha, Maharashtra, Goa, Kerala, Assam, Tamil Nadu, Sikkim, West Bengal and Jammu & Kashmir (Das et al., 2020; Shamal et al., 2025). Reports have shown that *M. nimmoniana* have a

potential alkaloid such as camptothecin that has an anti-cancer potential (Khan et al., 2013; Shwetha et al., 2020). Further medicinal properties for the treatment of malaria, HIV and fungal and bacterial infection have been recorded (Rather et al., 2018). Due to its therapeutic potential, the plant parts such as leaves, stems, roots and seeds are exported from India to countries like the USA, Japan and Spain (Das et al., 2020). Considering its therapeutic importance, a study has been conducted to find the qualitative and quantitative phyto-chemical analysis of *M. nimmoniana* and antimicrobial activity has been carried out to validate the therapeutic claims from different sources.

MATERIALS AND METHODS

Collection of plant sample

During the field survey in Deomali Hills of Koraput, Odisha, in November 2025, leaves of *M. nimmoniana* were collected (Fig.1) and photographs were taken for taxonomic identification using available published literature (Das et al., 2020) and e-herbarium specimens (IPNI). Plant parts were also collected and herbarium specimen was prepared with the necessary field information. Standard fungicide (2% $HgCl_2$) was applied to protect the herbarium specimen from fungal attack (Jain and Rao, 1976).

Fig. 1. *Mappia nimmoniana* plant parts: a) Apical stem bearing flowers, b) fruits, c) leaves d) collection of leaves in the field

Qualitative phytochemical analysis

Preparation of extracts

5 g of the coarsely ground leaves of *M. nimmoniana* was placed in a stoppered container with different solvents (50 ml each) and allowed to stand for 24 hours. The mixture was then strained, pressed and filtered. The process was repeated for the different selected solvents (distilled water, ethanol and n-hexane). The filtrate was then used for the qualitative analysis of the bioactive components (Abubakar and Haque, 2020; Jena et al., 2024; Marndi et al., 2024).

Secondary metabolites

Test for tannins

One ml of the leaf extract was taken and three to five drops of 10 % lead acetate solution were added. The white gelatinous precipitate formation confirms the presence of tannins.

Test for saponin

One ml of the leaf extract was taken and 1 ml of distilled water was added and shaken well. The formation of persistent froth confirms the presence of saponin.

Test for flavonoids

One ml of the leaf extract was taken and two ml of 2% NaOH solution and 3 to 4 drops of diluted HCl were added. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless. This change in colour confirms the presence of flavonoids.

Test for terpenoids

One ml of the leaf extract was taken and 5-6 drops of chloroform were added. It was then placed in the water bath for few minutes. Later, 5-6 drops of concentrated sulphuric acid were added. The appearance of a reddish-brown interface confirms the presence of terpenoids.

Test for phenolic groups

One ml of the leaf extract was taken and a few drops of 5% ferric chloride solution were added. The dark bluish-black appearance confirms the presence of phenolic compounds.

Test for reducing sugars

About 1 ml of the leaf extract was taken and 2 drops of Fehling's solution A followed by Fehling's solution B were added. It was then kept in the water bath for some time. The presence of red-orange precipitate confirms the presence of reducing sugar.

Test for steroids

One ml of the leaf extract was taken and 1 ml of chloroform and 1 ml of concentrated sulphuric acid were added to it. The presence of upper red and lower yellow colours with green fluorescence confirms the presence of steroids.

Test for alkaloids

One ml of the leaf extract was taken and 3 to 4 drops of Dragendorff's reagent were then added. The formation of a reddish-brown precipitate confirms the presence of alkaloids.

Quantitative phyto-chemical analysis

The quantitative analysis of the plant extracts was conducted using standard methods to assess their medicinal value.

Estimation of total tannin content

Extraction

0.5 mg of tannic acid was mixed with 1 ml of distilled water and from this solution, 5, 15, 25, 35 and 50 µl were taken in different test tubes. The volume was made to 1 ml. The 0.5 ml of Folin reagent and 2.5 ml of 20% sodium carbonate were added to each test tube. The mixed solutions were then shaken for 5 minutes in the dark conditions. The solution of each test tube was left for 40 minutes. Absorbance was then taken at 720 nm wavelength and a standard graph was plotted (Sadasivam and Manickam, 2009).

Estimation

5 mg of leaves was crushed and transferred into a 250 ml conical flask to which 75 ml of distilled water was added and boiled for 30 minutes. The whole solution was centrifuged at 2000 rpm for 20 minutes. Supernatant was taken in a 100 ml volumetric flask and made up to the volume. 1 ml of sample was taken from a 100 ml volumetric flask and 75 ml of distilled water, 5 ml of Folin reagent and 10 ml of 20% sodium carbonate were added. The volume was made up to 100 ml. After shaking for 5 minutes, the absorbance was taken at 720 nm wavelength. The amount of tannin was calculated from the tannic acid standard graph (Fig. 2).

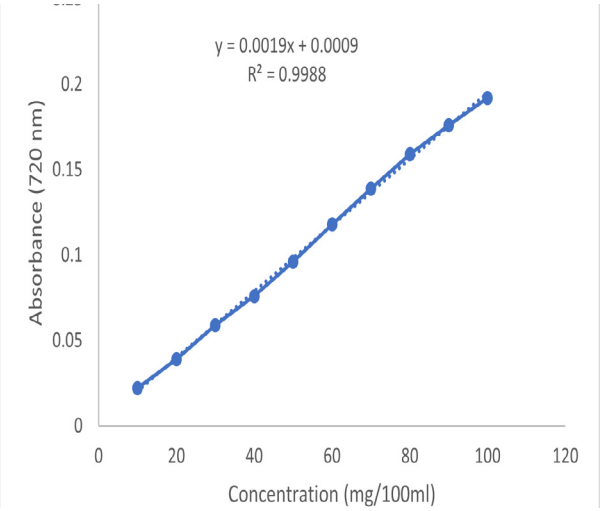

Fig. 2. Standard graph for tannic acid for the estimation of tannin

Estimation of total phenol content

Extraction

Three replicas of 0.5 g of leaves were taken and crushed with 60% methanol in a mortar and pestle. Samples were centrifuged 5 times at 5000 rpm for 20 minutes.

Estimation (in dark condition)

Seven test tubes were taken, including a blank and two test tubes for each replica. 0.1 and 0.2 ml of the sample were taken in each test tube except the blank. 60% methanol was added to each test tube to bring the volume up to 1 ml. 1 ml of 0.1 N HCl was added and allowed to stand for a few minutes. 1 ml of sodium nitrite molybdate mixture was added, shaken well and allowed to stand for a few minutes, then diluted with 5 ml of distilled water. After dilution, 2 ml of 1 N NaOH was added and allowed to stand for 20 minutes. Absorbance was taken at 515 nm wavelength. The amount of phenol present in the sample was calculated from the gallic acid standard graph (Swain and Hills, 1959; Fig. 3).

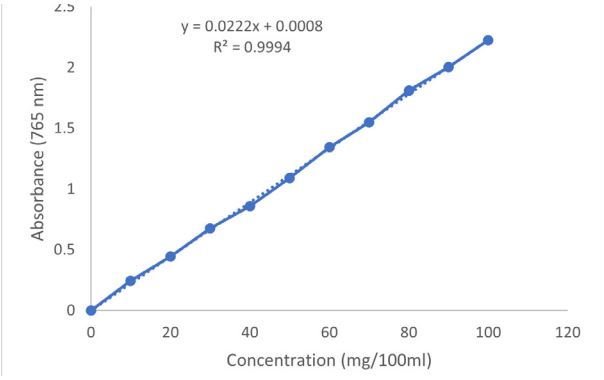

Fig. 3. Standard graph for gallic acid for the estimation of phenols

Estimation of flavonoids

Extraction

100 µl of the leaf aqueous extract was mixed with 100 µl of 5% sodium nitrite and allowed to stand for 5 minutes. Next, 150 µl of aluminium chloride was added and incubated for 6 minutes, followed by the addition of 200µl of 1 M NaOH. The total volume of the mixture was made up to 3 ml with distilled water and incubated for 15 minutes at room temperature in the dark for taking absorbance at 510 nm wavelength. The amount of flavonoid present in the sample was then calculated from the standard graph. The standard graph was prepared for quercetin (Chang et al., 2002; Shirazi et al., 2014; Raina and Misra, 2023; Fig. 4).

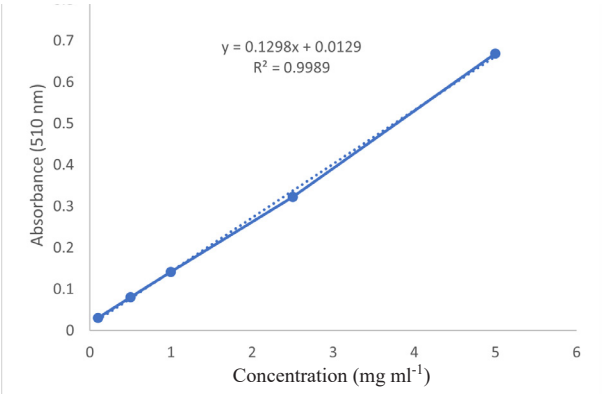

Fig. 4. Standard graph for quercetin for estimation of flavonoids

Estimation of saponin

The determination of total saponin was done by the method of Obadoni and Ochuko, (2001) with slight modifications. 5 g of crushed leaves was added to 100 ml of 20% aqueous ethanol and stirred for 30 minutes in a flask. It was heated for 4 h at 45 °C. The mixture was filtered using Whatman filter paper No. 1 and the residue was then extracted with an additional 100 ml of 25% aqueous ethanol. The combined extracts were concentrated and then transferred into a separator funnel and later extracted twice with 20 ml of diethyl ether. The ether layer was discarded, while the aqueous layer was kept and re-extracted with 30 ml n-butanol. The n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was evaporated. After evaporation, the sample was dried in the oven at 40 °C to a constant weight. The saponin content was then calculated.

Percentage (%) of saponin = (final weight of sample/initial weight of extract) x 100

Analysis for antibacterial activity

Soxhlet extraction

The Soxhlet extraction technique was used to assess antimicrobial activity. The method relies on repeated circulation of a solvent through reflux and siphoning to extract compounds from a solid sample. Dried leaf material of *M. nimmoniana* was placed in a thimble within the Soxhlet apparatus and a pure solvent was heated in a flask. The solvent vapour condensed, percolated through the sample and dissolved the phytochemicals. Once the solvent reached the siphon level, it returned to the flask and the cycle was repeated for 6-7 hours. The extract was then concentrated using a rotary evaporator to obtain the final plant extract (Suwari et al., 2017).

Disc diffusion assay

Disc Diffusion assay of the leaf extracts of *M. nimmoniana* was carried out according to the National Committee for Clinical Laboratory Standards (NCCLS). Inoculum was spread on the sterile nutrient agar plates with the sterile swabs of *E. coli* suspension. Different concentrations of the leaf extracts (6.25 mg ml^{-1}, 25mg ml^{-1}, 50mg ml^{-1} and 100mg ml^{-1}) were prepared using the different solvents. Whatman filter paper No. 1 was cut into 6mm disc pieces and allowed to suspend in the different concentrations of the

different leaf extracts for about 5 hours. Filter paper discs suspended in different concentrations of the solvent extracts were then placed on the nutrient agar plate with *E. coli* using a sterile forceps on the petri-plates with approximately equal distance. It was then placed for incubation at 37ºC for 24 hours. Ampicillin disc suspended in a 5mg ml^{-1} ampicillin solution was also placed as a positive control. Three replicates were carried out with each test organism. (Owusu et al., 2021).

Broth dilution assay (minimum inhibitory concentration)

Serial dilutions were made from the selected solvents of the leaf extract or stock solution (100 mg ml^{-1}). Different concentrations of the leaf extract (100 mg ml^{-1}, 50 mg ml^{-1}, 25 mg ml^{-1}, 12.5 mg ml^{-1} and 6.25 mg ml^{-1}) were prepared from the stock solution of the different solvents of leaf extracts, respectively. 100 µl of the bacterial inoculum and 500 µl of the leaf extract were added to the sterile nutrient broth. This incubation was carried out for different concentrations of the leaf extracts for the selected solvents. For positive control, inoculum was taken in sterile nutrient broth and as a negative control, sterile nutrient broth was taken and all were incubated at 37ºC for 24 hours. After 24 hours, turbidity was checked, compared and recorded (Owusu et al., 2021).

Analysis for antioxidant activity

Maceration method for plant extraction

1g of dried leaves was ground and placed in stoppered containers with 10 ml of solvent and was allowed to stand for 48 hours with occasional stirring. The reduced mixture was then strained and pressed. The process was followed with the two different selected solvents (distilled water and ethanol). It was then filtered. The filtrate was then dried in a hot-air oven to obtain crude extract and was diluted in 1% Dimethyl Sulfoxide to obtain the working extract. The technique (Abubakar and Haque, 2020) was followed with slight modifications.

Preparation of DPPH solution (0.1 mM)

2,2-Diphenyl-1-picrylhydrazyl (DPPH) stock solution was prepared by dissolving 3.94 mg of DPPH in 100 ml of methanol. A 0.1 mM working solution was then prepared by diluting 3 ml of the stock solution to 50 ml with methanol in a volumetric flask. The solution was wrapped in aluminium foil to protect it from light (Baliyan et al., 2022).

DPPH radical scavenging assay

Different concentrations of the leaf extracts were prepared in their respective solvents. DPPH solution was mixed with the extracts in a 1:1 ratio. Sample blanks containing extract and solvent (without DPPH) were used for background absorbance correction. 0.1 mM DPPH solution was used as a control, taking methanol as a blank. All the reaction mixtures were incubated in the dark at room temperature for 10 minutes and the absorbance was taken at 517 nm using a UV-Visible Spectrophotometer. The percentage of radical scavenging activity was calculated using the formula mentioned below. A percentage inhibition versus concentration graph was plotted using the resultant data for comparison and quercetin was taken as a standard (Majhi et al., 2026).

$$\% \text{ Inhibition} = \frac{\text{Absorbance}_{(control)} - \text{Absorbance}_{(sample)}}{\text{Absorbance}_{(control)}} \times 100$$

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The qualitative phytochemical screening of the leaf extract of *M. nimmoniana* revealed notable variations in the presence of bioactive compounds (Table 1). The aqueous extract showed the presence of tannins, saponins, terpenoids and alkaloids. The presence of saponins and terpenoids suggests that some moderately polar constituents are also soluble in aqueous medium. Ethanol extract exhibited saponins, terpenoids, reducing sugars and steroids. The presence of steroids, along with reducing sugars, indicates that ethanol is particularly efficient in extracting a diverse spectrum of bioactive constituents. The n-hexane extract, however,

showed only the presence of reducing sugars. Most bioactive compounds present in the leaf extract of *M. nimmoniana* are polar to moderately polar in nature. Camptothecin (CPT) is a well-known alkaloid obtained from *M. nimmoniana* plant parts that has been used as an anticancer agent (Ankad et al., 2015; Shamal et al., 2025). The compound is known to be present in leaves, stems, bark, roots and seeds. Other phytochemical compounds such as 9-methoxy camptothecin, mappicine, 9-amino camptothecin, topotecan, irinotecin and SN 38 are also reported from this plant (Nazeerullah et al., 2012; Khan et al., 2013; Godbole et al., 2023).

Table 1. Qualitative phytochemical analysis of *M. nimmoniana* leaves

Solvent used	Phytochemicals present
Aqueous extract	Tannin, saponin, terpenoid and alkaloids
Ethanol extract	Saponin, terpenoid, reducing sugar and steroid
n-hexane extract	Reducing sugar

Quantitative phytochemical analysis

The quantitative phytochemical analysis revealed the variation in the concentration of different bioactive compounds present in the leaf extract of *M. nimmoniana*. The tannin content was found to be the most abundant (22.15 mg/100 g) concentration. This high level of tannin correlates with a potential for antioxidant and antimicrobial activities, as tannins are known for their ability to precipitate proteins and inhibit microbial growth.

Table 2. Quantitative phytochemical analysis of *M. nimmoniana* leaves

Phytochemical compounds	Concentration
Tannin	22.15 mg/100 g
Phenol	2.171 mg/100 g
Flavonoid	0.458 mg/100 g
Saponin	5.994 %

The content of phenolic compounds was 2.171 mg/100 g, as recorded. Phenols also play a crucial role in antioxidant activity by scavenging free radicals and preventing oxidative stress. Flavonoids were present in significantly lower concentration (0.458 mg/100 g). Saponin content was detected with 5.994%, which demonstrates a considerable proportion in relation to other phyto-chemicals (Table 2). Saponins are also known for their surfactant properties and exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory and immune-modulatory effects.

Antibacterial activity

The ethanolic leaf extract of *M. nimmoniana* demonstrated good antibacterial activity as compared to the aqueous and n-hexane extract against *E. coli.* The ethanolic extract of 100 mg ml^{-1} concentration recorded the highest zone of inhibition ZI (20 ± 0.0), followed by the concentration of 50 mg ml^{-1} with ZI (19 ± 0.2). The least or negligible zone of inhibition was recorded by the n-hexane extract. The Minimum Inhibitory Concentration (MIC) was observed at 100 mg ml^{-1} (Table 3). Earlier studies have revealed the antibacterial activity against *Xanthomonas campestris* and *Aeromonas hydrophila,* which indirectly correlated to the therapeutic potential of diarrhoea and dysentery (Britto et al., 2014). Further, reports of antibacterial activity have been reported against *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans* (Shrivastava et al., 2023). Shrivastava and his team

in 2023 also reported that the methanol leaves extract of *M. Nimmoniana* shows good antibacterial activity against *Escherichia coli* (21 ± 0.0) mm and *Klebsiella pneumoniae* (23 ± 0.0) mm.

Table 3. Zone of inhibition produced by different solvents extracts of *M. nimmoniana* against *E. coli*

Test Samples	Zone of inhibition (mm)		
	n-hexane	Ethanol	Aqueous
100 mg ml^{-1}	7 ± 0.0	20 ± 0.0	15 ± 0.2
50 mg ml^{-1}	7 ± 0.1	19 ± 0.2	9 ± 0.0
25 mg ml^{-1}	7 ± 0.0	16 ± 0.1	8 ± 0.0
6.25 mg ml^{-1}	7 ± 0.0	15 ± 0.0	8 ± 0.0
Standard (Kanamycin) 5 mg ml^{-1}	30 ± 0.0	30 ± 0.0	30 ± 0.0

Antioxidant activity

The antioxidant activity of *M. nimmoniana* leaf extracts showed visible differences between the two tested solvents that were selected on the basis of the phytochemical screening results. The aqueous extract showed higher radical scavenging activity as compared to the ethanolic extract. At the lowest concentration of 0.125 mg ml^{-1}, the aqueous extract showed 54.04% inhibition, which is more than double that of the ethanolic extract (25.59%). This trend was observed with increasing concentration, reaching 58.26% for the aqueous extract and 39.42% for the ethanolic extract at 1 mg ml^{-1} (Fig. 5). The stronger activity of the aqueous extract can be linked to the presence of polar bioactive compounds such as the trigonelline (alkaloid) and pumiloside (monoterpene glycoside), both of which are water soluble and possess active radical scavenging potential (Khan et al., 2013). Presence of other compounds, many of which have significant cytotoxic activity but moderate antioxidant potency, like gypsogenin and enoxolone of the terpenoid class, camptoth ec in derivatives (alkaloid) and steroids like stigmast-5-en-3-β, 7-α-diol and sitosteryl-β-D-glucoside, may contribute to the radical scavenging activity observed in the ethanolic extract (Godhaniya and Ganatra, 2021). On the other hand, the standard quercetin showed very high radical scavenging activity even at much lower concentrations. At 8.33 µg ml^{-1}, it already showed 96.85% inhibition, increasing only slightly to 97.5% at 66.67 µg ml^{-1}. This indicates the potent antioxidant capacity of quercetin as a pure bioactive compound (Qi et al., 2022), far exceeding that of the crude plant extracts. The minimal increase across concentrations also shows a near-saturation effect, where quercetin achieves maximal radical scavenging efficiency even at the lowest dose.

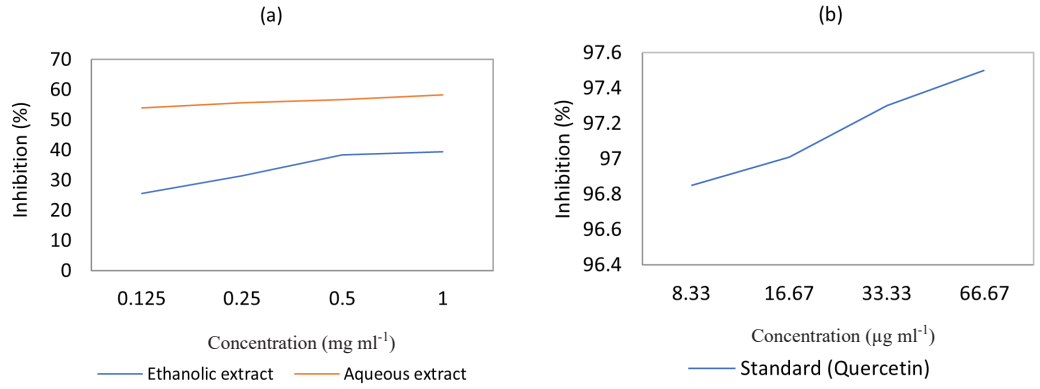

Fig. 5 (a, b). Percentage inhibition against concentrations showing DPPH free radical scavenging activity of (a) aqueous and ethanolic leaf extracts of *Mappia nimmoniana* and (b) quercetin standard

CONCLUSION

The present study demonstrates that the bioactive compounds soluble in the polar solvents show various bioactivities, including antibacterial and antioxidant activity. The study is supported by the presence of secondary metabolites such as tannin, saponin, terpenoids and alkaloids. The abundant presence of alkaloids is also linked to the antioxidant activity and may possess anti-cancer potential. Present study may help to identify bioactive compounds and for future drug development, keeping in view the sustainable extraction of plant parts and conservation of *M. nimmoniana.*

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Additional records of foliicolous lichens to the state of Kerala, India

S.A. ZACHARIAH[1*], S. JOSEPH[2] AND K.S. ASHNAMOL[1]

[1] *Postgraduate and Research Department of Botany, Mar Thoma College, Tiruvalla - 689 103, Kerala, India*

[2] *Forest Botany Department, Forest Ecology and Biodiversity Conservation Division, KSCSTE - Kerala Forest Research Institute, Peechi - 680 653, Thrissur, Kerala, India*

* *sonia@mtct.ac.in*

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ABSTRACT

Five species of foliicolous lichens growing on tropical evergreen trees in Pathanamthitta and Alappuzha districts of Kerala are reported as additions to the lichen flora of the state. *Coenogonium isidiiferum* (Lücking) Lücking, *Microxyphiomyces vainioi* (R. Sant.) Xavier-Leite, M. Cáceres & Lücking, *Porina andamanensis* Upreti & Ajay Singh, *Strigula antillarum* (Fée) Müll. Arg. and *Strigula subelegans* Vain. were identified and briefly described based on morphological and anatomical characters. With about 25 foliicolous lichens previously reported from Kerala, this article significantly contributes to the state's lichen flora, adding five new records that promote our understanding of the region's biodiversity.

Key words: Additional records, crustose thallus, foliicolous lichens, Kerala

INTRODUCTION

Foliicolous lichens, those that grow on the surfaces of green foliage of vascular plants. In India, 154 species and 185 taxa have been documented (Singh & Pinokiyo, 2014; Upadhyay et al., 2015; Subrahmanya & Krishnamurthy, 2015a, 2015b, 2016b; Gupta & Sinha, 2014, 2016; Jagadeesh Ram, 2015; Subrahmanya & Krishnamurthy, 2016a; Rashmi & Rajkumar, 2015; Randive et al., 2017, 2019; Haridas et al., 2021; Jagadeesh Ram & Sinha, 2022; Sinha et al., 2024).

Despite that Kerala harbouring a rich lichen diversity exceeding as much as 780 species (Sinha et al., 2024), foliicolous lichens remain still understudied. Publications on the state's lichen biota primarily focus on corticolous (bark-dwelling) and saxicolous (rock-dwelling) species (Kumar et al., 1999, 2000; Haridas et al., 2010, 2012, 2014; Zachariah et al., 2018, 2020; Anilkumar et al., 2022). A comprehensive study on foliicolous lichens is lacking, with only sporadic records for about 25 species documented (Singh & Sinha, 2010; Haridas et al., 2021; Sinha et al., 2024). This has to be given more emphasis considering Kerala's abundance of tropical evergreen trees with perennial leaves, an ideal habitat for foliicolous lichen growth.

During a study on lichen flora, conducted in the Pathanamthitta district of Kerala during 2014 to 2018, as well as in Sharngakavu (sacred grove) in the Alappuzha district in 2024, the authors collected and further identified five foliicolous lichen species new to the state. These findings pave the way for further research on foliicolous lichens and contribute to a better understanding of their distribution in Kerala and across the country.

MATERIALS AND METHODS

The specimens examined in this study were collected from Pathanamthitta and Alappuzha districts of Kerala and are housed in the herbaria of RHK (Regional Herbarium of Kerala, S.B. College, Changanacherry, Kottayam) and KFRI (Kerala

Forest Research Institute, Peechi, Thrissur). Morphological and anatomical characters were studied using a stereo zoom microscope (Olympus SZ61) and a light microscope (DM 2500) equipped with a camera and image analysis software. Hand-cut sections were mounted in distilled water and lactophenol cotton blue. All measurements were made from material mounted in distilled water. Plant identification was performed by examining the taxonomic characters and referring to the keys and descriptions referring to the literature (Aptroot et al., 2003; Pinokiyo & Singh, 2004; Jagadeesh Ram & Sinha, 2018; Sinha et al., 2024). Nomenclature was updated following Index Fungorum (2025).

RESULTS AND DISCUSSION

A total number of five species mentioned below were identified as new records for Kerala.

1. Coenogonium isidiiferum **(Lücking) Lücking,** Lichenologist 33(3): 201 (2001). ≡*Dimerella isidiifera* Lücking, Lichenologist 31(4): 367 (1999); Family: Coenogoniaceae (Fig. 1).

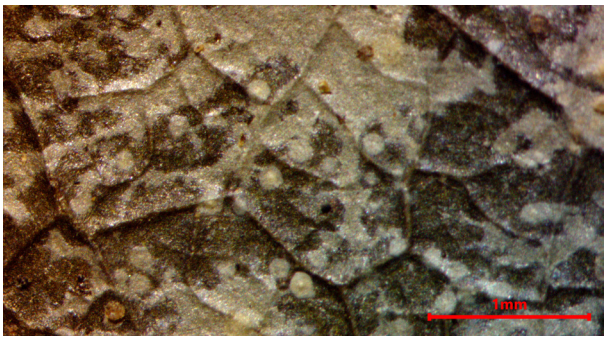

Fig. 1. *Coenogonium isidiiferum*

Description

Thallus crustose, epiphyllous, smooth, lacinate, greyish-greenish; thallodiscs numerous, disc-shaped, exfoliating thallodiscs leaving ±rounded holes in the thallus. Photobiont *Trentepohlia*, cells angular, arranged in irregular plates. Ascomata not seen.

Specimen examined

India, Kerala, Pathanamthitta district, Adoor, Koodal, elev. 74 m, 9°8′16″N 76°52′38″E, 07 November 2018, S.A. Zachariah L0449 (RHK).

Notes

The species is a new record to Kerala state and is previously known from Andaman & Nicobar Islands as *C. disciforme* Papong et al., (Jagadeesh Ram & Sinha, 2018). Kalb et al., (2016) synonymised *C. disciforme* under *C. isidiiferum*.

2. Porina andamanensis **Upreti & Ajay Singh,** Bot. J. Linn. Soc. 94(3): 399 (1987); Family: Trichotheliaceae (Fig. 2).

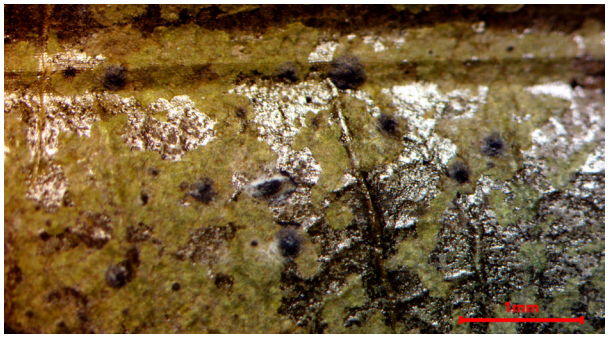

Fig. 2. *Porina andamanensis*

Description

Thallus crustose, epiphyllous, greenish grey, smooth, matt, occurs in small irregularly continuous scattered patches, hypothallus not seen. Photobiont *Phycopeltis*, cells arranged in non-radiating plates. Ascomata perithecia, very few, black; periderm differentiated into an involucrellum and excipulum, involucrellum black-brownish, spreading laterally, 5-20 µm thick in upper part, excipulum dark coloured- brownish; paraphyses simple, colourless, 70-90 µm high. Asci 8-spored, clavate, 58-75 ×10-15 µm. Ascospores hyaline, transversely 7- septate, oblong or fusiform, 22-35 × 5-6 µm.

Specimen examined

India, Kerala, Alappuzha district, Venmony, Sharngakavu, elev. 35 m, 3 May 2024, N 9°14’ 37.7844” E 76° 37’ 52.2876”, Ashnamol KA 25-41264c (KFRI).

Notes

This species is a new record to Kerala state and is previously known from Andaman & Nicobar Islands (Sinha et al., 2024).

3. *Strigula antillarum* (Fée) Müll. Arg., Bot. Jahrb. 6: 379 (1885). ≡*Melanophthalmum antillarum* Fée, Essai Crypt. Exot. (Paris): xciv, c (1825); Family: Strigulaceae (Fig. 3).

Fig. 3. *Strigula antillarum*

Description

Thallus crustose, epiphyllous, whitish- green, dispersed into small, scattered, ± rounded patches. Photobiont *Cephaleuros*, cells rectangular to irregular in shape. Ascomata perithecia exposed, black; ostiole inconspicuous; periderm differentiated into outer involucrellum and inner excipulum, involucrellum blackish, 20-30 µm thick, excipulum pale brown; paraphyses simple, colourless, 40-100 µm high. Asci 8-spored, clavate, 50-90 × 8-10 µm. Ascospores hyaline, transversely 1-septate, fusiform, with acute ends, 12-24 × 3-5 µm. Pycnidia aggregated at the centre, black, wart shaped; macroconidia hyaline, 1-septate, bacillar, 10-16 × 3-5 µm.

Specimens examined

India, Kerala, Pathanamthitta district, Adoor, Parakkodu, elev. 61 m, N 9°9′11″ E 76°45′49″E, 07 November 2018, S A Zachariah L0451 (RHK); ibid., Alappuzha district, Venmony, Sharngakavu, elev. 35 m, 3 May 2024, N 9°14’ 37.80” E 76° 37’ 51.92”, Ashnamol KA 25-41268, 25-41274, 25-41277, 25-41281, 25-41282, 25-41284, 25-41287 (KFRI).

Notes

This species is a new record to Kerala state and is previously known from Andaman & Nicobar, Arunachal Pradesh, Assam, Goa, Meghalaya, Manipur, Sikkim, and W.B. (Sinha et al. 2024).

4. *Strigula subelegans* Vain., Ann. Acad. Sci. Fenn., Ser. A. 19: 23 (1923); Family: Strigulaceae (Fig. 4).

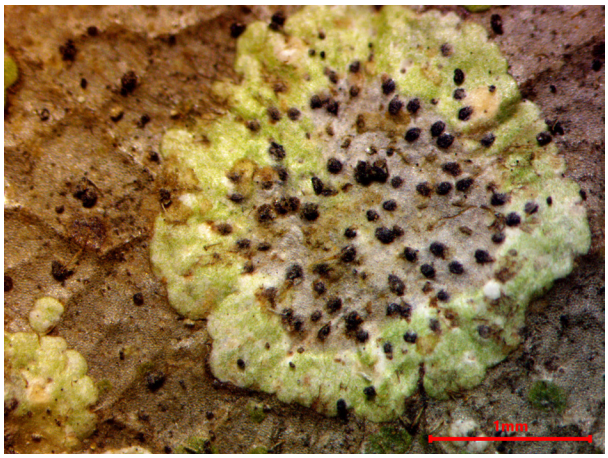

Fig. 4. *Strigula subelegans*

Description

Thallus crustose, epiphyllous, pale greenish-greyish-whitish, ± circular, sometimes irregular scattered patches, margin entire and lobed, lobes short. Photobiont *Phycopeltis*, cells rectangular, in irregular plates. Ascomata perithecia, semi-immersed, black, ostiole grey-white; periderm divided into outer involucrellum and inner excipulum, involucrellum blackish, 15-25 µm thick, excipulum colourless; paraphyses simple, septate, colourless, 80-120 µm high. Asci 8-spored, clavate, 45-90 × 10-15 µm. Ascospores hyaline, fusiform, 1-septate, 14-26 × 3-6 µm. Pycnidia immersed, blackish; macroconidia hyaline, 1-septate, bacillar, 10-18 × 2-4 µm.

Specimen examined

India, Kerala, Pathanamthitta district, Konni, elev. 49 m, 9°13′54″ N 76°50′39″E, 26 October 2018, S A Zachariah L0332 (RHK).

Notes

This species is a new record to Kerala state and are previously known from Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Goa, Karnataka, Manipur, Meghalaya, Nagaland, Sikkim, Tamil Nadu, and West Bengal (Sinha et al., 2024).

5. *Microxyphiomyces vainioi* (R. Sant.) Xavier-Leite, M. Cáceres & Lücking, Mycol. Progr. 22(12, no. 88): 12 (2023). ≡*Tricharia vainioiR*. Sant., Symb. Bot. Upsal. 12(1): 382 (1952); Family: Gomphillaceae (Fig. 5).

Fig. 5. *Microxyphiomyces vainioi*

Description **:** Thallus crustose, epiphyllous, small, smooth, greyish-green-greyish white, with numerous short sterile setae, setae black, stiff, slightly swollen at base, tapering upwards, up to 1.2 mm long. Photobiont green algae, cells rounded. Ascomata apothecia, sessile, rounded, very few observed. Asci 1-spored, clavate to ovoid, 40-70 × 15-30 µm. Ascospores hyaline, muriform, oblong-ellipsoid, 37-60 × 13-27 µm. Pycnidia not seen.

Specimen examined : Kerala, Pathanamthitta district, Puthukulam, elev. 139 m, 9°17′54″ N 76°50′31″E, 12 November 2018, S.A. Zachariah L0452 (RHK).

Notes **:** Xavier-Leite et al. (2023) resurrected the genus Microxyphiomyces to accommodate the Tricharia vainioi clade. This species is a new record to Kerala state and is previously known from Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Karnataka, Mizoram, Nagaland, Sikkim, Tamil Nadu, and West Bengal (Sinha et al., 2024).

Flourishing populations of foliicolous lichens, known for their sensitivity to environmental conditions, can serve as bio-indicators of an area's ecosystem. Entirely dependent on the surrounding atmosphere for survival and growth, these lichens have life spans closely connected to that of their host leaves of trees in-situ. Consequently, they exhibit rapid life cycles and respond quickly to changes in their environment (Lücking, 1997; Randive et al., 2017). The luxuriant lichen growth observed on many tropical crop land trees in Kerala presents a valuable opportunity for further investigation. Further, studying these lichens holds its potential for developing bio-monitoring programmes in the region.

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Baseline inventory of herpetofauna from the Naneghat lateritic plateau in the Western Ghats, Maharashtra, India

A. DOLAS*, S.S. MAHARANA AND A. SHA. ARUN

Wildlife SOS, Junnar, Maharashtra, India

**akashdls8@gmail.com*

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ABSTRACT

Amphibians and reptiles are vital components of ecological networks, acting as both predators and prey, and serving as sensitive bioindicators of environmental health. The Western Ghats, a UNESCO World Heritage Site and a global biodiversity hotspot, harbour exceptional levels of herpetofaunal endemism; yet, many microhabitats remain poorly explored. Here we present the first systematic assessment of herpetofaunal diversity on the Naneghat plateau (~750 m asl), Maharashtra, India. Field surveys were conducted during the monsoon seasons from June 2020 to June 2025 using standardized visual encounter surveys, random walks, and active microhabitat searches. A total of 53 species were recorded, comprising 9 anurans, 13 lizards, and 31 snakes, across 18 families. According to the IUCN Red List (2025), 44 species are listed as Least Concern, four have not yet been assessed, while *Varanus bengalensis*, *Uropeltis bicatenata*, *Gongylophis conicus*, and *Eryx johnii* are categorized as Near Threatened. Of the recorded species, 34 are protected under the Wildlife (Protection) Amendment Act, 2022. This study provides a baseline inventory for this underexplored landscape and underscores the need for long-term monitoring and site-specific conservation strategies.

Key words: Baseline inventory, conservation, herpetofauna, Naneghat plateau

INTRODUCTION

Herpetofauna (amphibians and reptiles) play a crucial role in ecosystem stability by regulating invertebrate populations, contributing to nutrient cycling, and serving as indicators of habitat quality (Gibbons et al., 2000). Globally, herpetofaunal populations are experiencing severe declines, primarily due to habitat degradation, climate change, over exploitation, and emerging diseases (Wake and Vredenburg, 2008).

India supports a rich assemblage of herpetofauna, with 454 amphibian species (Dinesh et al., 2023) and 778 reptile taxa (Mohapatra et al., 2024). The Western Ghats alone host over 227 reptile species, nearly half of which are endemic (Myers et al., 2000). However, northern sectors of the Western Ghats, particularly the basaltic plateaus and lateritic outcrops remain under represented in ecological surveys, despite being highly vulnerable to anthropogenic pressures including agriculture, infrastructure expansion, and tourism (Aphale et al., 2019; More et al., 2025).

The study aims to document the herpetofaunal diversity of the Naneghat plateau, Maharashtra. Specifically, it seeks to: (1) establish a baseline inventory of species, (2) assess their conservation status under the IUCN Red List and the Indian Wildlife (Protection) Act (as amended), and (3) highlight the ecological and conservation significance of this plateau habitat within the Western Ghats biodiversity hotspot.

MATERIALS AND METHODS

Study area

The Naneghat plateau is located in Pune district, Maharashtra (19°16′45.63″N, 73°41′19.96″E to 19°17′53.29″N, 73°40′26.37″E), at an elevation of ~750 m and covering ~0.75 km². The landscape is characterized by basaltic outcrops, scrub vegetation, forest patches, paddy fields, and steep escarpments, with annual rainfall ranging from 700 to 1200 mm. The plateau is flanked by sacred groves and reserve forests (Rahangdale and Rahangdale, 2014) as shown in Fig. 1.

Survey methods

Field surveys were conducted during the southwest monsoon between June 2020 and June 2025. Standardized visual encounter surveys (Heyer et al., 1994), random trail walks (Lambe, 1984), and active microhabitat searches (Rolfe and McKenzie, 2000) were employed across diurnal (1600–1900 h) and nocturnal (1900–0300 h) periods. Surveys focused on key microhabitats, including streamside vegetation, rocky crevices, and arboreal niches.

Species were photographed and identified using standard taxonomic keys. (Daniel, 2002; Whitaker and Captain, 2008; Das and Das, 2017). Geographic coordinates were recorded using the NoteCam mobile application and mapped in QGIS (v3.44), shown in Fig. 1. Conservation status was assessed using the IUCN Red List (2025) and the Wildlife (Protection) Amendment Act, 2022.

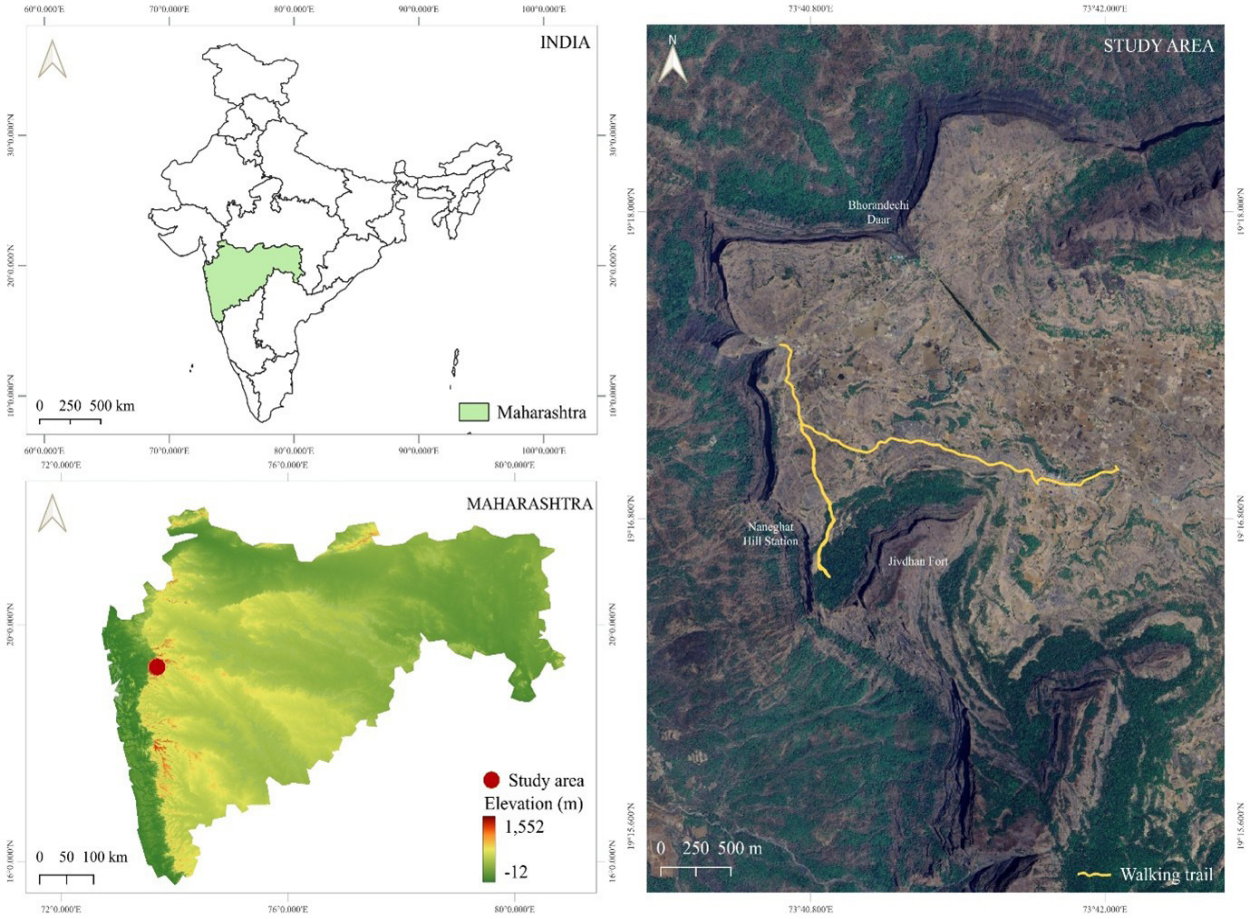

Fig. 1. Location and topography of the study area showing major landmarks and the walking trail (yellow line) in Maharashtra, India

RESULTS AND DISCUSSION

A total of 53 herpetofaunal species belonging to 18 families were documented from the Naneghat plateau (Table 1), comprising 9 anurans (5 families, 9 genera; shown in Fig. 2), 13 lizards (5 families, 9 genera as shown in Fig. 3), and 31 snakes (8 families, 23 genera; shown in Fig. 4). Among amphibians, Dicroglossidae was the most represented family (4 species), followed by Rhacophoridae (2 species), while Ranidae, Ranixalidae, and Bufonidae were represented by one species each. Reptiles comprised 44 species across 13 families, with Colubridae being the most diverse (14 species), followed by Gekkonidae (6 species); Elapidae, Scincidae, Natricidae, Uropeltidae, and Viperidae (3 species each); Erycidae, Agamidae, and Typhlopidae (2 species each); Varanidae, Chamaeleonidae, and Sibynophiidae (1 species each). Among the recorded taxa, *Varanus bengalensis*, *Chamaeleo zeylanicus*, *Daboia russelii*, *Naja naja*, *Fowlea piscator*, *Ptyas mucosa*, and *Eryx johnii* have the highest legal protection under Schedule I, while two anuran species, one lizard, and 24 snake species are listed under Schedule II of the Wildlife (Protection) Amendment Act, 2022.

Table 1. Annotated list of herpetofauna recorded in Naneghat plateau, India, during the study period

Class/Family	Name of Species	WPA	IUCN
AMPHIBIA: ANURA			
Dicroglossidae			
	Paddy field cricket frog (*Minervarya agricola*)	NS	LC
	Indian bullfrog (*Hoplobatrachus tigerinus*)	II	LC
	Indian skittering frog (*Euphlyctis cyanophlyctis*)	II	LC
	Maskey's burrowing frog (*Sphaerotheca maskeyi*)	NS	LC
Ranidae			
	Wide-spread fungoid frog (*Hylarana bahuvistara*)	NS	LC
Ranixalidae			
	Matheran leaping frog (*Indirana leithii*)	NS	LC
Rhacophoridae			
	Maharashtra bush frog (*Raorchestes ghatei*)	NS	LC
	Indian tree frog (*Polypedates maculatus*)	NS	LC
Bufonidae			
	Common Indian toad (*Duttaphrynus melanostictus*)	NS	LC
REPTILIA: SQUAMATA (lizards)			
Chamaeleonidae			
	Indian chameleon *(Chamaeleo zeylanicus*)	I	LC
Agamidae			
	Oriental garden lizard (*Calotes versicolor*)	NS	LC
	Roux's forest lizard (*Monilesaurus rouxii*)	NS	LC

Gekkonidae			
	Aaron bauer's house gecko (*Hemidactylus aaronbaueri*)	NS	LC
	Dakota's leaf-toed gecko (*Hemidactylus triedrus*)	NS	LC
	Amarasinghe's house gecko (*Hemidactylus amarasinghei*)	NS	NE
	Spotted leaf-toed gecko (*Hemidactylus maculatus*)	NS	LC
	Deccan spotted gecko (*Cyrtodactylus deccanensis*)	II	LC
	Common house gecko (*Hemidactylus frenatus*)	NS	LC
Varanidae			
	Bengal monitor lizard (*Varanus bengalensis*)	I	NT
Scincidae			
	Lined supple skink (*Riopa lineata*)	NS	LC
	Keeled Indian mabuya (*Eutropis carinata*)	NS	LC
	Bronze mabuya (*Eutropis macularia*)	NS	LC
REPTILIA: SQUAMATA (snakes)			
Viperidae			
	Common bamboo pit viper (*Craspedocephalus gramineus*)	NS	LC
	Indian saw-scaled viper (*Echis carinatus*)	II	LC
	Russell's viper (*Daboia russelii*)	I	LC
Elapidae			
	Common krait (*Bungarus caeruleus*)	II	LC
	Spectacled cobra (*Naja naja*)	I	LC
	Slender coral snake (*Calliophis melanurus*)	II	LC
Natricidae			
	Asiatic water snake (*Fowlea piscator*)	I	LC
	Buff striped keelback (*Amphiesma stolatum*)	II	LC
	Green keelback (*Rhabdophis plumbicolor*)	II	LC
Colubridae			
	Beddome's cat snake (*Boiga beddomei*)	II	LC
	Forsten's cat snake (*Boiga forsteni*)	II	LC
	Common cat snake (*Boiga trigonata*)	II	LC
	Northern vine snake (*Ahaetulla borealis*)	II	NE
	Slender-nosed vine snake (*Ahaetulla oxyrhyncha*)	II	NE
	Oriental ratsnake (*Ptyas mucosa*)	I	LC
	Common wolf snake (*Lycodon aulicus*)	II	LC
	Travancore wolf snake (*Lycodon travancoricus*)	II	LC
	Indian trinket snake (*Coelognathus helena*)	II	LC

	Common bronzeback tree snake (*Dendrelaphis tristis*)	II	LC
	Streaked kukri snake (*Oligodon taeniolatus*)	II	LC
	Tillack's kukri snake (*Oligodon tillacki*)	II	NE
	Günther's racer (*Platyceps gracilis*)	II	DD
	Banded racer (*Platyceps plinii*)	NS	LC
Uropeltidae			
	Bombay shieldtail *(Uropeltis macrolepis*)	II	LC
	Elliot's shieldtail *(Uropeltis ellioti*)	II	LC
	Bicatenate shieldtail *(Uropeltis bicatenata*)	II	NT
Erycidae			
	Common sand boa *(Gongylophis conicus*)	II	NT
	Red sand boa *(Eryx johnii*)	I	NT
Typhlopidae			
	Brahminy blind snake *(Indotyphlops braminus*)	II	LC
	Beaked worm snake *(Grypotyphlops acutus*)	II	LC
Sibynophiidae			
	Dumeril's black-headed snake *(Sibynophis subpunctatus*)	II	LC

WPA = Wildlife (Protection) Amendment Act, 2022: I = Schedule I, II = Schedule II, NS = Non Schedule. IUCN (International Union for Conservation of Nature and Natural Resources), Red List of Threatened Species (IUCN 2025); NE = Not Evaluated, DD = Data Deficient, LC = Least Concern, NT = Near Threatened

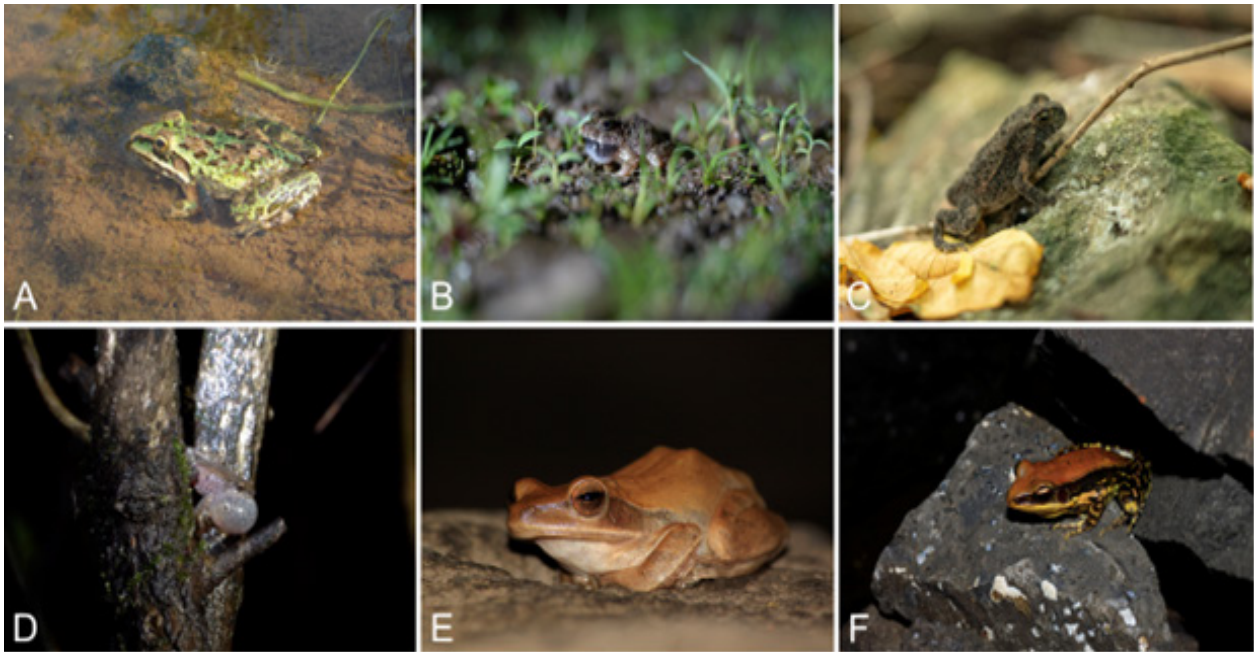

Fig. 2. A. *Hoplobatrachus tigerinus*, B. *Minervarya agricola*, C. *Duttaphrynus melanostictus*, D. *Raorchestes ghatei*, E. *Polypedates maculatus*, F. *Hylarana bahuvistara*

Fig. 3. A. *Chamaeleo zeylanicus*, B. *Varanus bengalensis*, C.*Cyrtodactylus deccanensis*, D. *Hemidactylus triedrus*, E. *Eutropis macularia*, F. *Riopa lineata*

Fig. 4. A. *Craspedocephalus gramineus,* B. *Echis carinatus,* C. *Bungarus caeruleus,* D. *Naja naja,* E. *Calliophis melanurus,* F. *Rhabdophis plumbicolor,* G. *Boiga beddomei,* H. *Ahaetulla borealis,* I. *Lycodon travancoricus,* J. *Platyceps gracilis,* K. *Uropeltis bicatenata,* L. *Eryx johnii,* M. *Sibynophis subpunctatus,* N. *Oligodon tillacki,* O. *Amphiesma stolatum*

The high herpetofaunal diversity recorded from the Naneghat plateau is notable given its limited geographic extent and highlights the ecological value of plateau ecosystems in the northern Western Ghats. The presence of threatened and legally protected species further underscores the conservation importance of this landscape. Basaltic plateaus and lateritic outcrops, though often overlooked in regional conservation planning, provide a mosaic of microhabitats that

support diverse reptile and amphibian assemblages (Aphale et al., 2019).

The observed species composition is consistent with broader patterns of herpetofaunal diversity and endemism reported from the Western Ghats (Myers et al., 2000). However, the dependence of many species on specialized microhabitats suggests heightened vulnerability to land-use change, habitat modification, and unregulated tourism. Such pressures are increasingly evident across plateau systems in the northern Western Ghats and may disproportionately affect habitat-specialist taxa.

These findings indicate the need for targeted conservation interventions, including long-term population monitoring, improved habitat protection through integration into regional conservation frameworks, community-based awareness initiatives emphasizing the ecological roles of herpetofauna, and regulation of unsustainable anthropogenic activities. Recognition of plateau ecosystems as conservation-relevant habitats is essential for safeguarding herpetofaunal diversity in the northern Western Ghats.

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Tuberculous pericarditis in a captive sloth bear (*Melursus ursinus*): A case report

S. ILAYARAJA*, A. SHA. ARUN, N.K. ADHITHYAN, P. ACHARYA, S.S. MAHARANA AND M.V. BAIJURAJ

Agra Bear Rescue Facility, Wildlife SOS, Agra, India

**ilasuvet@gmail.com*

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ABSTRACT

Tuberculous pericarditis (TBP), an uncommon extrapulmonary manifestation of *Mycobacterium tuberculosis* complex infections, is rarely documented in bears. A 25-year-old sick captive male sloth bear (*Melursus ursinus*) was presented to the Agra Bear Rescue Facility for necessary investigation. The animal exhibited progressive weakness, respiratory distress, drowsiness, and arrhythmias, culminating in cardiovascular failure. Ante-mortem investigations, including Ziehl–Neelsen staining, MycoPac dual kit, and thoracic radiography, revealed acid-fast bacilli, seropositivity for *Mycobacterium* spp. leading to pneumonic changes, and cardiac enlargement. Post-mortem examination revealed granulomatous pericarditis with necrosis, epithelioid cell granulomas, histiocytic infiltration, and acid-fast bacilli. Supportive care along with antitubercular therapy was provided. This case highlights the rarity of TBP in ursids, underscores the diagnostic challenges in captive wildlife, and emphasizes the importance of routine screening, quarantine, and stress-reducing management strategies to mitigate disease risk.

Key words: Diagnosis, histopathology, preventive measures, sloth bear, tuberculous pericarditis

INTRODUCTION

Tuberculosis (TB) is a transmissible zoonotic disease and remains one of the leading causes of morbidity and mortality worldwide. It is caused by members of the *Mycobacterium tuberculosis* complex (MTBC), which includes *M. tuberculosis, M. bovis* and related species. While human cases predominate, TB also affects domestic and wild animals, which may serve as reservoirs or spillover hosts at the human–animal interface (Teppawar et al., 2018; Marinaik et al., 2022). The sloth bear (*Melursus ursinus*), a vulnerable species native to the Indian subcontinent (Dharaiya et al., 2016), is particularly susceptible to infectious diseases under captive conditions. Several studies have documented TB in sloth bears through histopathology, bacterial isolation, molecular characterization, and drug-susceptibility testing of *M. tuberculosis* isolates in both free-ranging and captive populations (Hedau and Kamdi, 2016; Menon et al., 2021; Marinaik et al., 2022; Sharma et al., 2022). In humans, tuberculous pericarditis (TBP) accounts for approximately 1–2% of pulmonary TB cases and typically manifests as pericardial effusion, constrictive pericarditis, or cardiac tamponade. Diagnosis is often challenging due to non-specific clinical signs (Lucero et al., 2022; Wang et al., 2022). While pulmonary TB is relatively well characterized in ursids, extrapulmonary involvement, particularly of cardiac structures, is exceedingly rare. To date, cardiac tuberculomas have been reported in dogs (Szaluś-Jordanow et al., 2016). Given the rarity of this condition, early diagnosis and intervention are critical for prognosis and disease management. Here, the authors described this particular case of TBP in a captive geriatric sloth bear, detailing the clinical presentation, ante-mortem and post-mortem diagnostic findings, pathological features, and therapeutic approach.

MATERIALS AND METHODS

Clinical examination

A 25-year-old rehabilitated geriatric male sloth bear maintained at the Agra Bear Rescue Facility, Wildlife SOS, was presented with reduced appetite, mucopurulent ocular discharge, progressive weakness, respiratory distress, lethargy, and irregular cardiac rhythms. A detailed clinical evaluation was performed, which included thoracic radiography using a portable digital radiography unit (EP-CORSA 2.4, Epsilon Healthcare Solutions Pvt. Ltd., India) with a Focus 35C detector (Carestream, India), to assess pulmonary and cardiac structures.

Laboratory investigations

Hematological parameters were evaluated using an automated hematology analyzer (ProCyte Dx, IDEXX Laboratories, Inc.) and serum biochemical analyses were conducted with a clinical chemistry analyzer (Catalyst One, IDEXX Laboratories, Inc.). Serological screening for tuberculosis was performed using the MycoPac dual kit for the detection of antibodies against *Mycobacterium* spp.

Therapeutic management

The animal was isolated and treated with conventional antitubercular drugs in combination with oral multivitamin supplementation. Supportive therapy was continued; however, the clinical course remained progressive.

Necropsy and histopathology

The animal eventually succumbed to the disease, and a complete necropsy was performed in accordance with standard protocols. Representative tissue samples were collected and fixed in 10% neutral buffered formalin for histo-pathological examination. Ziehl–Neelsen staining was used on tissue samples to confirm the presence of acid-fast bacilli (AFB).

RESULTS AND DISCUSSION

Despite supportive therapy with anti-tubercular drugs and multivitamins, the clinical course remained unfavorable, culminating in cardiovascular failure. Hematological analysis revealed microcytosis with anaemia, while serum biochemistry indicated reduced blood urea nitrogen level (Table 1). Thoracic radiography demonstrated diffuse pneumonic changes with focal nodular opacities in the pulmonary lobes and cardiomegaly with indistinct margins suggestive of pericardial involvement (Fig. 1). Serology (MycoPac dual kit) was positive for *Mycobacterium* spp. (Fig. 2). Necropsy revealed granulomatous inflammation with caseating tubercles on the pericardium, and disseminated lesions including splenomegaly, hepatomegaly, pulmonary nodules, renal infarcts, and lymphadenopathy (Fig. 3). Histopathology of pericardial tissue demonstrated necrosis, epithelioid granulomas, histiocytic infiltration, and multinucleated giant cells, while Ziehl–Neelsen staining confirmed presence of acid-fast bacilli (Fig. 4), thereby establishing a diagnosis of tuberculous pericarditis with systemic dissemination.

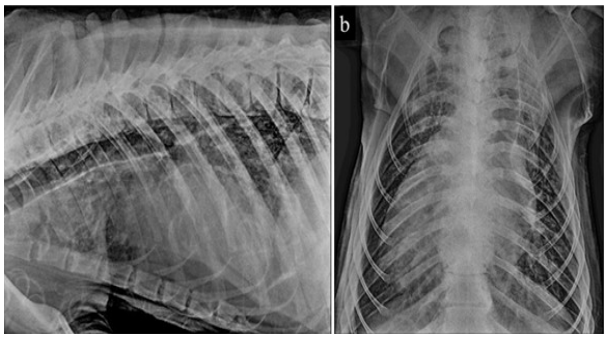

Fig. 1. Thoracic radiographs showing (a) diffuse pneumonic changes in the pulmonary lobes with nodular lesions and (b) cardiomegaly with indistinct cardiac margins

The case underscores the diagnostic challenges posed by extra-pulmonary tuberculosis in wildlife, particularly when clinical signs are nonspecific. Comprehensive evaluation integrating clinical examination, hematology, biochemistry, radiography, and serology was essential for establishing the diagnosis. The findings emphasize the need for routine tuberculosis screening, quarantine and biosecurity protocols in captive settings, and supportive therapy with immune modulators is also essential to minimize stress thereby enhancing the wellbeing.

Table 1. Representative hemato-biochemical values

Measurand (unit)	Results
Red blood cell count (M μL^{-1})	4.68
Haemoglobin (g dL^{-1})	12.2
Mean corpuscular volume (fL)	64.5
Mean corpuscular haemoglobin (pg)	26.1
Mean corpuscular haemoglobin concentration (g dL^{-1})	40.4
White blood cell count (K μL^{-1})	8.73
Neutrophils (K μL^{-1})	5.99
Lymphocytes (K μL^{-1})	1.70
Monocytes (K μL^{-1})	0.28
Eosinophils (K μL^{-1})	0.70

Measurand (unit)	Results
Basophils (K μL^{-1})	0.06
Platelets (K μL^{-1})	583
Glucose (mg dL^{-1})	65
Blood urea nitrogen (mg dL^{-1})	3
Creatinine (mg dL^{-1})	1.0
Total protein (g dL^{-1})	6.7
Albumin (g dL^{-1})	2.2
Globulin (g dL^{-1})	4.5
Alanine aminotransferase (U L^{-1})	< 10
Alkaline phosphatase (U L^{-1})	24
Lactate dehydrogenase (U L^{-1})	845

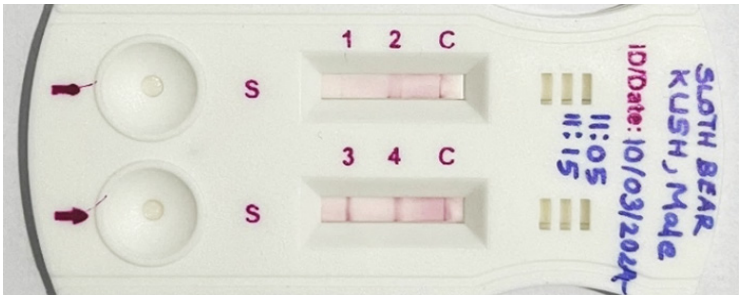

Fig. 2. Serology (MycoPac dual kit) revealed TB sero-positive against *T. mycobacteria*

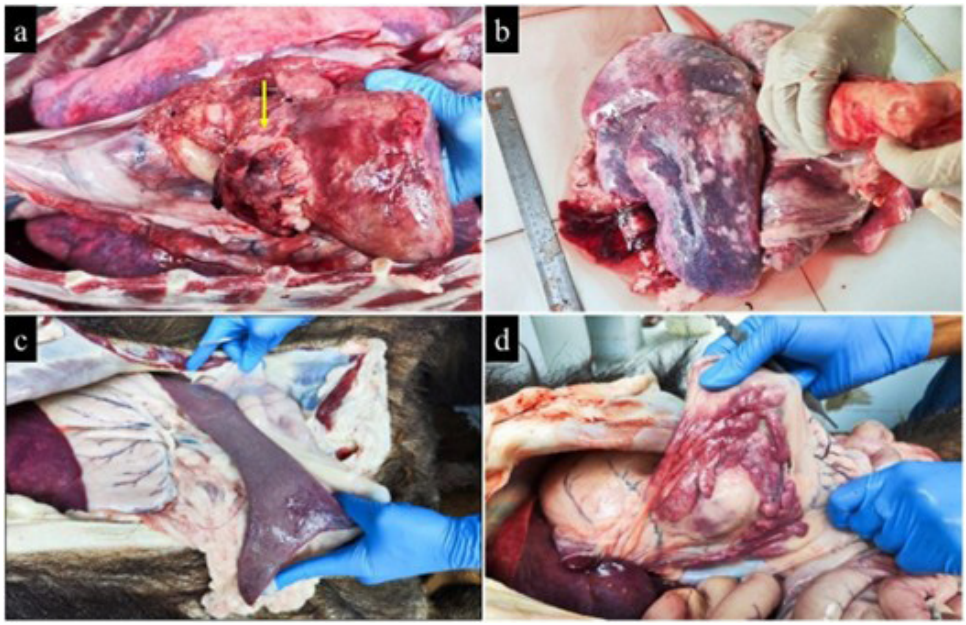

Fig. 3. PM lesions demonstrating (a, b) granulomatous inflammation with caseating tubercles on the pericardium, (c) disseminated lesions including splenomegaly and hepatomegaly, and (d) renal infarcts

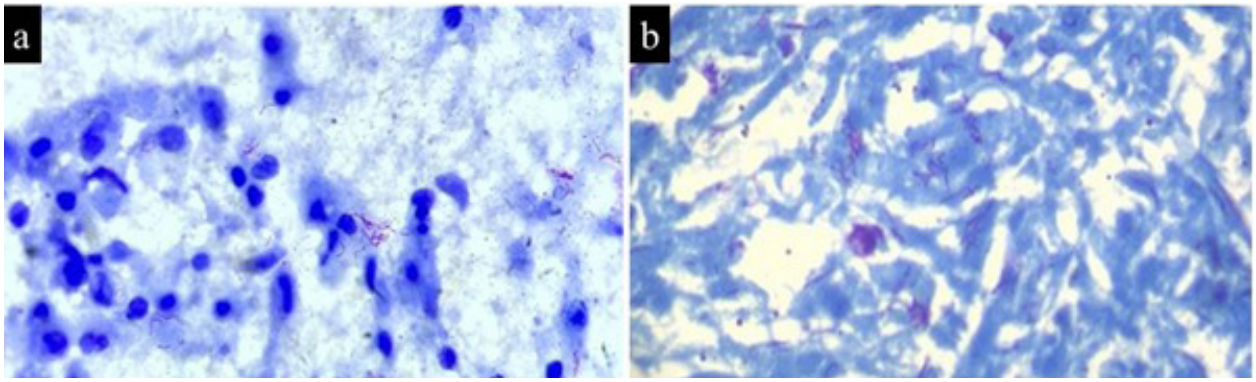

Fig. 4. Ziehl-Neelsen stain revealed presence of acid-fast bacilli in (a) lung and (b) affected pericardial tissue under magnification (X100)

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New record of Pallas's gull [*Ichthyaetus ichthyaetus* (Pallas, 1773)] in the Brahmani river, Bonai forest division, Odisha, India

L.K. PATRA[1], S.K. JENA[2*], S. PAIRA[3], N.C. PALEI[4], B.P. RATH[5] AND M. GIRI[6]

[1,2,3&6]*Office of the Divisional Forest Officer, Bonai forest division, Sundargarh, Odisha, India*

[4]*1398/3427, Sasanpadia, Old Town, Bhubaneswar, Odisha, India-751002*

[5]*B-3, CTTC, CAMPUS, PO- KITT, Khordha, Bhubaneswar, Odisha, India-751024*

**sanjaybiologist@gmail.com*

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ABSTRACT

The Pallas's gull, *Ichthyaetus ichthyaetus* (Pallas, 1773) or the Great black-headed gull, happens to be the world's largest black-headed gull and the third largest species of gull in the world, followed by the great black-backed gull. It is the most spectacular fish-eating predator among the world's gulls. A survey was conducted in and around Brahmani river at Bonai forest division, Odisha. The water level of Brahmani was low during the winter season. However, for the first time the authors sighted these migratory bird species in the Brahmani basin under Bonai forest division. The present data on the current status and distribution of the Pallas's gull in the Brahmani river stretch has been documented. Previously the Pallas's gull has been reported from different parts of Odisha. But in recent times, due to lack of food and impact of climate changes, these birds might have changed their distribution pattern. These birds were found for the first time in Bonai under the present survey. Authors herewith suggest that a long-term monitoring is required to determine and establish the exact status, distribution of these species at Bonai, Odisha.

Keywords: Bonai forest division, Brahmani river, migration, Odisha, Pallas's gull

INTRODUCTION

India is recognized as one of the world's megadiverse countries, occupying only about 2.4% of the global land area while supporting approximately 7–8% of the world's recorded biodiversity. The country is home to more than 45,000 plant species and 91,000 animal species (Swain and Samantarai, 2024). Among these, birds constitute an important component of biodiversity and play a vital role in maintaining ecosystem functions. They contribute significantly to pollination, seed dispersal, pest control, and nutrient cycling, thereby supporting ecological balance and providing substantial benefits to both natural ecosystems and human well-being (Nazneen et al., 2001). Owing to their sensitivity to environmental changes, birds are also considered effective indicators of ecosystem health and habitat quality.

India supports a rich avifaunal diversity, with approximately 1,340 bird species recorded from the country, representing nearly 13% of the world's bird species (Ali & Ripley, 1987; Grimmett et al., 2012). Of these, around 310 species are associated with wetland ecosystems and depend on these habitats for

feeding, breeding, nesting, or migration (Kumar et al., 2005). Wetlands are among the most productive ecosystems and provide critical habitat for a wide range of resident and migratory birds. However, the increasing anthropogenic pressures, including habitat degradation, pollution, encroachment, and unsustainable resource use, have severely affected wetland ecosystems across India (Prasad et al., 2002). Such disturbances can alter habitat quality and availability, thereby influencing the composition, abundance, and distribution of bird communities (Reginald et al., 2007).

The Pallas's gull *Ichthyaetus ichthyaetus* (Pallas, 1773) is one of the largest and most spectacular fish-eating predators among the world's gulls. The breeding area of the species is located entirely in the Palearctic, inside the continent. By the beginning of the 21st century, the range of the Pallas's gull extended from the Black and Azov Seas in the west to the Great Lakes in Mongolia and Uryugnor in China in the east. Non-breeding individuals were mainly found in the breeding area of the species and to the south (including south of the southern border of the Palearctic region).

The rare migratory Pallas's gull belongs to the family Laridae. It is classified as Least Concern under the IUCN Red List. A frequent seasonal yearly journey taken by birds to meet the challenges of food availability, weather or habitat has been known as migration (Berthold et al., 2001). In the last decade, the importance of understanding the pattern and timing of migration for the conservation of migratory bird species has greatly increased. (Boere and Stroud, 2006; Mundkur, 2007). There are around 1377 bird species recorded in the Indian subcontinent, 370 migratory bird species that visit in the winter (Birdlife International 2016). Among the water birds ducks and geese only represent about 85% of the migrant populations to the Indian subcontinent (Kumar et al., 2003, 2005). Only 2.8% of work has been conducted on bird migration in India (Narwade et al., 2012). The present study documents the first sightings and record of Pallas's gull [*Ichthyaetus ichthyaetus* Pallas, 1773)] at Bonai, Odisha.

It is a large piscivorous gull and one of five species of gulls that are included in the family Laridae. This species breeds in colonies in marshes and islands from southern Russia to Mongolia. It is migratory; wintering in the eastern Mediterranean, Arabia and India. According to Olsen and Larsson (2004), Pallas's gull is a large migratory gull widely distributed across Europe and Asia, with wintering populations occurring in parts of the Indian subcontinent. The species is generally associated with large rivers, reservoirs, lakes, wetlands, estuaries, and coastal habitats. Breeding adult has a black head with a thin incomplete crescent around the eye and a red-and-black-tipped bill. Non-breeding adults retain a partial "hood" of patchy black on the back of the head. Young birds also have this dark patch, though it is much smaller in size (https://ebird.org/species/gbhgul2). These birds are predatory in nature, feeding on fish, crustaceans, insects and small mammals.

Pallas's gull is one of the species covered under the Agreement on the Conservation of African-Eurasian Migratory Waterbirds (AEWA). However, in parts of Odisha, there have been sighting of these birds including monitoring and documentation of the species (Changder et al., 2015). Against this background, the authors tried their best to document the current status and distribution pattern of Pallas's gull in Bonai.

MATERIALS AND METHODS

Study area

The Bonai forest division is located between 21º 39′–22º 8′ N latitude and 84º 30′–85º 23′ E longitude in the northwestern part of Odisha, eastern India. The division covers an area of approximately 2934.21 km² within Sundergarh district (Fig. 1). It is bounded to the north by Jharkhand state and the Rourkela forest division, to the east by Keonjhar forest division and Deogarh forest division, and to the west and south by Bamra forest division and Deogarh forest division. The division falls under the Rourkela forest circle of Sundergarh district. The Bonai forest division is administratively divided into seven forest ranges: Bonai, Kuliposh, Tamra, Jarda, Sole, Barsuan, and Koira. Ecologically,

the division is part of the Chotanagpur Plateau within the Deccan Peninsular Biogeographic Zone (Rodgers and Panwar, 1988). The region's terrain, climate, and vegetation types provide suitable habitats for a wide variety of flora and fauna, including a rich diversity of avian species.

The mean daily temperatures of winter range from 5ºC to 20ºC and that of summers range from 30ºC to 45ºC. There are three distinct seasons that is Summer; March to June, Rainy; July to October and winter; November to February. The rainfall of the division and the nearby areas varies from 1000 mm to 1800mm. According to Champion and Seth (1968), the forest under Bonai forest division belong to following types: North Indian tropical moist deciduous forest, Northern tropical dry deciduous forest, and northern tropical semi-evergreen forests. In sal forests (*Shorea robusta*), the top or canopy storey is typically dominated by sal trees, and the common associate species in the upper storey include a mix of other tall, emergent, and semi-emergent trees. Some of the frequent associates are: *Terminalia alata, Madhuca indica, Diospyros melanoxylon, Lagerstroemia parviflora, Pterocarpus marsupium, Dalbergia paniculata, Anogeissus latifolia, Syzygium cumini, Adina cordifolia, Mitragyna parviflora, Terminalia bellirica* etc.

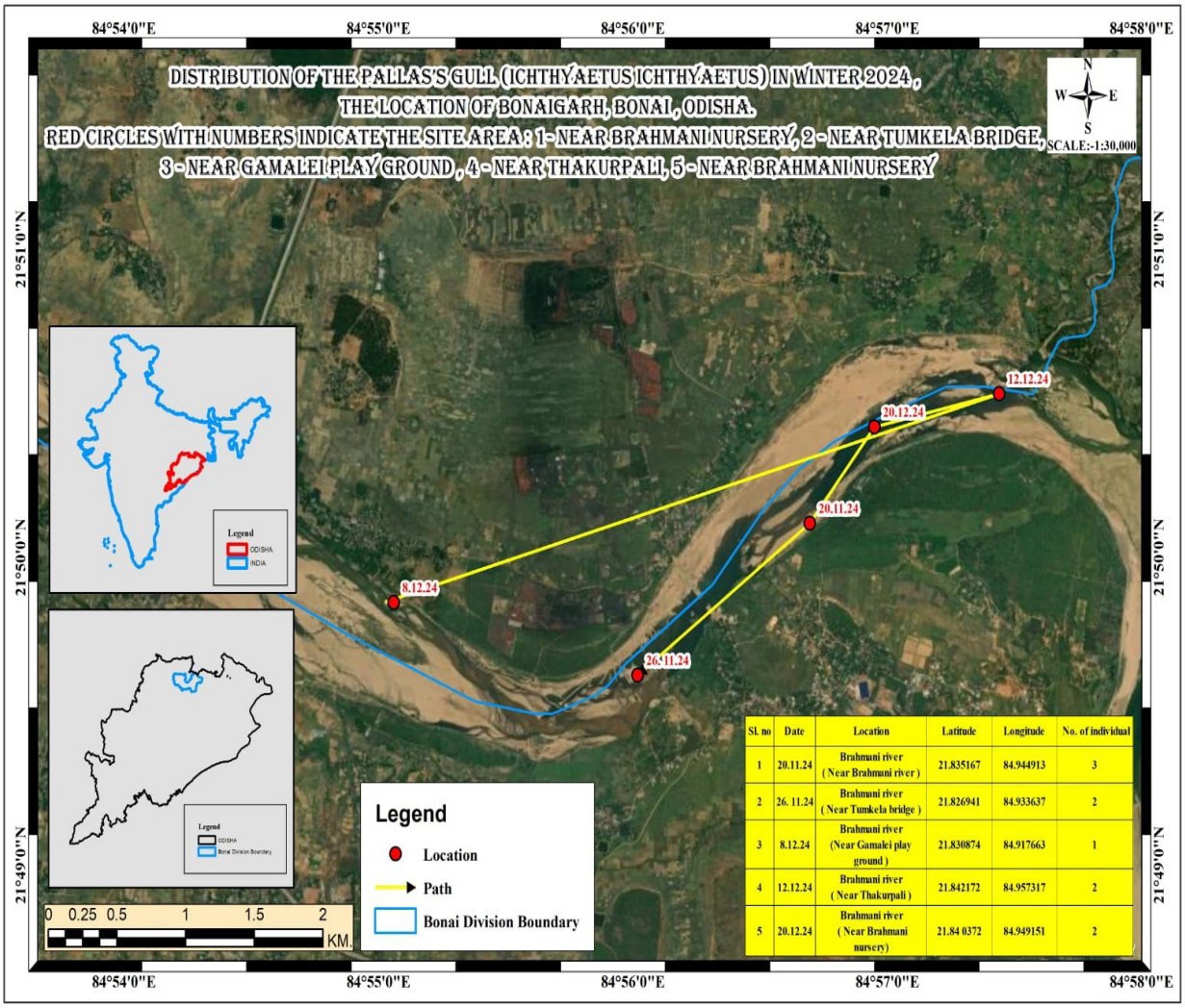

Fig. 1. Distribution of Pallas's gulls in 5 different sites at Brahmani river, Bonai, Odisha, India

Field survey and data collection

The authors made routine field trips around Brahmani river for structured data collection from 20th November to 20th December 2024. The authors came across the particular bird during their survey, photographed and later identified it as Pallas's gull with the help of standard literature (Ali, 1996; Neelakantan et. al., 1993) and expert consultation. These particular birds were never sighted so far in this region as per the data collected from the localities. Hence, it was decided to record the sightings with photographs to analyze its distribution pattern. Direct observation was made using a binocular and photographed by a camera (NIKON-COOLPIX P1000) the observation and data were collected per day for two months from 01.11. 2024 to 31.12. 2024 (morning 06.00 -10.00 and evening 4.00-6.30 PM). Most of the observations were carried out from a distance of 50 - 600 m. Care was taken not to disturb the activity of these birds in the study area. For secondary inputs, the authors recorded the location of the places and also the number of individual species found to interpreted the density pattern of the species and also the ratio of young and adult.

RESULTS AND DISCUSSION

Bonai forest division is recognized as a biodiversity-rich landscape; however, its avifaunal diversity remains inadequately documented (Singh et al., 2007). Previous studies have largely focused on noteworthy species records rather than comprehensive assessments of bird diversity. Mishra et al. (2008) and Nath et al. (2011) reported the first occurrence of the Cinereous vulture (*Aegypius monachus*) from the region, highlighting the ecological significance of Bonai forest division as an important habitat for both resident and migratory bird species.

The present study documents the first wintering record of the Pallas's gull (*Ichthyaetus ichthyaetus*) from Bonai forest division, Odisha. During routine monitoring surveys, individuals of the species were recorded from multiple locations along the Brahmani river system within the division, enabling a preliminary assessment of its local distribution (Fig. 2). Although Pallas's gull has been reported from several parts of Odisha, including a recent sighting at Mangalajodi Wetland, Khordha, reported by Bharatendra Singh Parihar on 27 December 2024. As such no previous records of Pallas's gull sighting are available from the Brahmani River system of Bonai forest division, Sundargarh district, Odisha. Pallas's gull is a fully migratory species, although immature individuals may occasionally remain within wintering grounds throughout the year. The absence of earlier records from this region and the recent observations suggest a possible shift or expansion in the wintering distribution of the species. Such changes may be influenced by factors including habitat alteration, anthropogenic disturbances, food availability, and changing climatic conditions. Continued monitoring of riverine habitats and wetland ecosystems in the region is therefore essential to better understand the distribution, ecology and conservation requirements of this species.

The study further indicates that rivers are not the only lotic ecosystems facilitating the spread of the species toward eastern Odisha. The increasing number of fish ponds and aquaculture areas around Bonai also appear to provide suitable feeding habitats for gulls. In the context of climate warming, fish-rich ponds, wetlands, and associated water bodies along the Brahmani River may now be acting as important ecological drivers influencing the modern distribution and expansion of the Pallas's gull beyond its historical range. The analysis highlights the growing ecological importance of the Brahmani river and associated wetlands in supporting the present distribution of the Pallas's gull in Bonai. The species was observed utilizing multiple rivers, lakes, reservoirs, and water bodies within the landscape. It is expected that additional records may emerge in the near future if birdwatchers, researchers, and forest officials continue regular monitoring and documentation of migratory water birds across Odisha. Although the current findings are preliminary, they provide valuable baseline information regarding the status and distribution of Pallas's gull in the region. Future studies focusing on seasonal movement, habitat preference, and population trends will be

essential for understanding the species' response to environmental changes. Despite the relatively stable global population of the species, continuous monitoring remains important for understanding its changing wintering distribution and potential breeding ecology in eastern India. Regular surveys of major rivers, wetlands, lakes, reservoirs, and fish ponds throughout the year are necessary to better understand migratory movement patterns and habitat use by wintering water birds.

Fig. 2. The Pallas's gull sited in specific location of Brahmani river basin - A. Brahmani nursery; B.Tumkela bridge; C. Gamalei playground; D. Thakurpali; E. Brahmani nursery

The development and maintenance of suitable wetland habitats may help support the expanding wintering range of migratory gulls while also reducing potential ecological conflicts among different migratory bird species in the future. At present, limited information is available regarding the detailed distribution pattern of the Pallas's gull in Odisha. Therefore, systematic documentation of sightings and habitat associations will be crucial for future conservation planning and wetland management. The major outcomes of this study include preliminary estimates of the current status and distribution of Pallas's gull along the Brahmani river system and adjacent wetlands within Bonai forest division (Table 1).

Table 1. Status and abundance of Pallas's gulls (*Ichthyaetus ichthyaetus*) in Brahmani river (Nov – Dec 2024)

Sl. No.	Date	Location	GPS Coordinates	No. of individual
1	20.11.2024	Brahmani river (near Brahmani nursery)	21.835167 N 84.944913 E	3
2	26.11.2024	Brahmani river (near Tumkela bridge)	21.826941 N 84.933637 E	2
3	08.12.2024	Brahmani river (near Gamalei playground)	21.830874 N 84.917663 E	1
4	12.12.2024	Brahmani river (near Thakurpali)	21.842172 N 84.957317 E	2
5	20.12.2024	Brahmani river (near Brahmani nursery)	21.840372 N 84.949151 E	2

CONCLUSION

The study is the first report of the distribution of gull species at Bonai, Odisha. The conservation of river ecosystems, feeding habitats, and breeding sites, coupled with continued monitoring, is essential for the long-term survival and management of aquatic bird populations. Routine surveys helped in finding out the present status and occurrence. This study is very significant in describing the current status of gulls and their present habitat that have not been explored much and addressed properly so far. This study will benefit the wild fragmented migratory aquatic bird population and have a significant conservation value. Additional scientific interdisciplinary research is essential for saving both wetland habitats and the aquatic bird diversity that rely on them in future. These findings also put hope in the field of conservation, showing the potential and possibility of habitat expansion to support thrive the cross country migratory avian species.

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