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Aquaculture at Risk in a Changing Climate: A Review of the Future of Aquatic Food Production

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Abstract

Aquaculture, although experiencing substantial expansion in recent times for fulfilling the global food demand of enlarging population, is facing mounting challenges because of changing climate. Elevating temperatures, acidification in ocean, increased salinity and unpredictable weather patterns threaten the sustainability of aquaculture operations impacting water quality, fish health and aquaculture settings. For instance, due to elevation in water temperature, it generates stressed conditions for fish and directs them towards diseases. Elevated sea levels, extreme weather events and altered precipitation levels can also create vulnerable environment for aquaculture facilities particularly near the coasts and low-lying regions. Thus, it is important to point out the climate-related problems in aquaculture so that its growth can be sustained and can be able to supply food and nutrition in non-stop way. This means finding ways of acclimatizing the aquaculture practices to changing circumstances, such as selecting species that are more resilient to warmer waters or designing aquaculture systems that can withstand climate related challenges like elevated sea levels. Thus, climate change is not just a puzzle for aquaculture; it is a pressing issue that requires attention and innovative approaches to maintain consistent food availability amid global change.

Keywords : Aquaculture, Fisheries, Climate change, Food security, Sustainability

Aquaculture and fisheries perform an important character in upholding nutrition and food availability and helps in poverty elimination and ultimately strengthen the economies of nations (Hodar *et al.*, 2021). Over the last three decades, the aquaculture industry has gained remarkable progress and is expected to continue increasing and expanding, from its current share of 46 to 53 per cent by 2030 (FAO, 2020). This expansion is crucial due to the need of providing food amid projections that place the global population at close to 10 billion by 2050 (UNESCO, 2020). Nevertheless, climate change poses a significant threat to the stability and expansion of aquaculture production (Barange *et al.*, 2018 and Dabbadie *et al.*, 2018). Climate change is now widely acknowledged as a major threat to sustainable agricultural systems, with serious implications for global food security-one of the central targets set forth by the UN's (United Nations) Sustainable Development Goals for 2030 (Hamdan *et al.*, 2015; Kandu, 2017; Barange *et al.*, 2018; Rajesh *et al.*, 2024 and Nandini & Venkataramana, 2024). The consequences of changing climate on aquaculture productions could be extreme, including reduced productivity, decreased food supplies, malnutrition, unemployment, income loss and broader negative effects on national economies (Vannuccini *et al.*, 2018).

Changes in climate affect aquaculture and fisheries production across the globe, because climate plays an important part in determining fish populations and

fish distribution (Lehodey *et al.*, 2006). Changes in climatic conditions, including alterations in oceanic circulation patterns, elevated ocean temperatures, shifts in precipitation regimes and more frequent extreme climatic events - can disrupt species distribution and in turn, impact the productivity of fish populations (Fleming *et al.*, 2014; Blanchard *et al.*, 2017 and Troell *et al.*, 2017). These disruptions often lead to reduced availability or accessibility of key habitats, adversely affecting fish stock yields (Perry *et al.*, 2005; Nilsson *et al.*, 2009 and Pankhurst & Munday, 2011). In some cases, these climatic changes can render previously suitable environments uninhabitable, potentially causing population declines or even local or global extinctions (Roessig *et al.*, 2004 and Ganachaud *et al.*, 2011).

This review article explores the impact of climate change on aquaculture production by examining the complex interactions between shifting climate variables and threats to sustainable aquaculture. In doing so, it emphasizes the urgent need for developing and implementing adaptation and mitigation strategies to protect aquatic ecosystems, support global food security and sustain the livelihoods of communities that depend on these vital resources.

Overview of and Reasons Behind Climate Changes

The Industrial Revolution, after its emergence at the 18th century, has fuelled global economic growth but unfortunately, it has also a significant negative effect in accelerating climate change and global warming (Kandu, 2017). While the words 'global warming' and 'climate change' are usually applied interchangeably, it must be keep to consider that global warming is just one aspect of the broader issue of climate change (El-Sayed and Kamel, 2020). The forthcoming outcomes of these phenomena are horrific for earth and its inhabitants and have generated enough concerns (Chatterjee and Sanyal, 2023).

Climate change, a interlinked complicated phenomena, is mainly triggered due to myriad of factors, including both natural phenomenas and anthropogenic activities (EPA, 2014) (Fig. 1). While

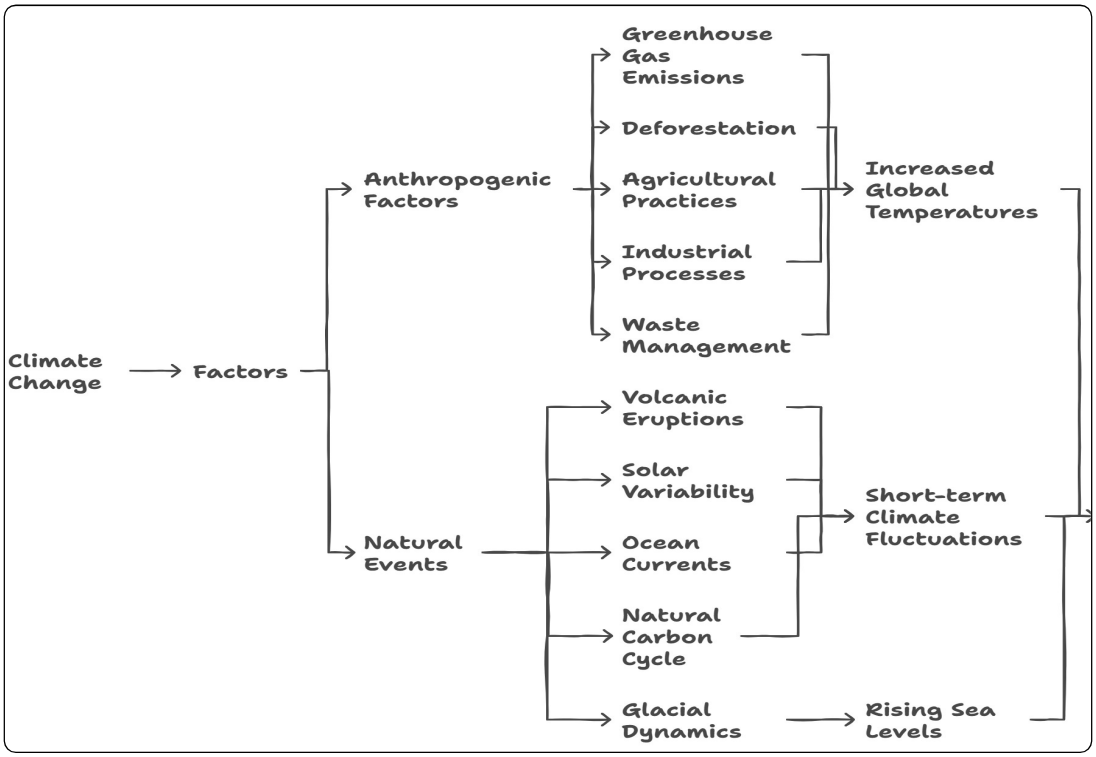

Fig. 1 : Climate change influences and consequences

natural calamities like solar activity, volcanic eruptions can be reason for alterations in the Earth's climate, the fingerprints of man-made contributions to global warming are mostly worrying (Tett *et al.*, 2002; Stott, 2003 and El-Sayed & Kamel, 2020). The constant dependency of mankind on fossil fuels for their daily activities has led to the release of copious concentrations of CO and other atmospheric heat-retaining compounds into the atmosphere, creating an enlarging crisis of air pollution (EPA, 2014 and IPCC, 2019). This energy imbalance and man-made pollution has triggered the accumulation of heat, resulting in the warming of the planet (Chatterjee and Sanyal, 2023).

The future scenarios of the warmer planet are far-reaching and beyond imagination that include either intensified or reduced rainfall, more powerful storms and cyclones, along with notable impacts on uprising sea temperatures and air quality (IPCC, 2019). Moreover, the scary prediction by the Intergovernmental Panel on Climate Change (IPCC) on elevation of worldwide temperature by 1.5°C during current century cannot be negligible, with ever-lasting hazards for the planet (IPCC, 2018). Due to predicted rise in global temperature, one significantly rising concern is the upliftment of sea levels, which is going to happen due to the loss of terrestrial ice and the heat-driven expansion of seawater (Hansen *et al.*, 2016). These situation is projected to bring in a rising seas expected between 9 and 88 centimeters between 1990 and 2100 (Gregory *et al.*, 2001 and Hansen *et al.*, 2016).

Deforestation, often induced by agricultural expansion, urbanization and industrialization, plays another major part to the elevation in atmospheric carbon dioxide levels (FAO, 2011). Apart from carbon emissions, deforestation is the reason behind changes in global and regional climate patterns, such as lengthen droughts (Strasser *et al.*, 2014). These environmental alterations trigger a chain of impacts across ecosystems, wildlife and plant species, (El-Sayed and Kamel, 2020).

Climate Change and its Implications for Fisheries and Aquaculture

The multidimensional aspects of climate variability and its effects on aquaculture such as changes in oceanic conditions, altered precipitation levels, rising temperatures all has straightforward effects on the physiology and behaviour and ultimately survival of fish (Handisyde *et al.*, 2006). For instance, when temperature fluctuates, it can disorganize normal growth patterns, affecting the overall development of fish. Thus reproductive functionalities may also be hampered as a result, altering in spawning behaviours and reduced breeding success. Furthermore, the distribution patterns of many fish species are changing in response to changing thermal conditions in oceanic regions, resulting altered geographical ranges over which they are traditionally found (Handisyde *et al.*, 2006). Beyond these direct consequences, climate change has far-reaching and indirect consequences affecting the productivity, integrity and biodiversity of ecosystems that supply food and habitat essential for sustaining fish populations. For instance, variations in thermal conditions and ocean chemistry may affect the population levels and spatial patterns of plankton, which ultimately disastrous for the food resources availability for many fish species. In Addition, disruptions in the physical structure of marine habitats because of climate-induced incident like Rising sea levels and increased ocean acidity may reduce the accessibility of appropriate habitats and spawning sites for these species. These complicated scenarios can spread through the food web, ultimately downgrading the abundance and health of commercially important fish stocks (Handisyde *et al.*, 2006) (Fig. 2).

Influence of Climate-Related Thermal Fluctuations on Aquatic Environments and Aquaculture : Fish, being ectothermic creatures, are reasonably vulnerable to temperature variations generated by changed climate, notably in tropical and subtropical regions (Sae-Lim *et al.*, 2017 and Adhikari *et al.*, 2018). Lengthened periods of temperature has direct impacts on physiology, growth pattern, metabolism, feeding behaviour of mature individuals as well as their larvae and juveniles (Table 1).

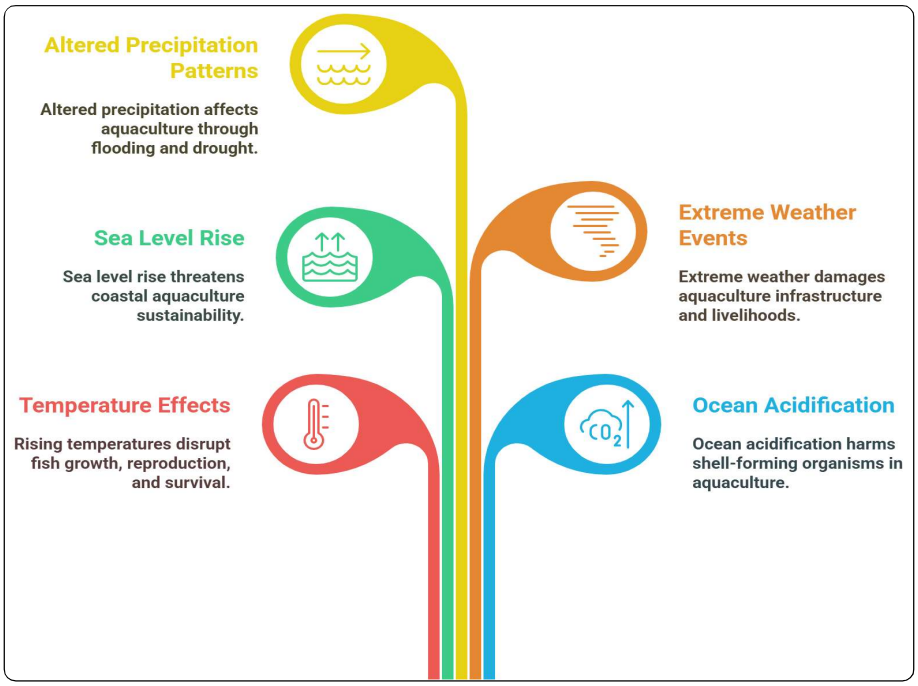

Fig. 2 : Climate change's multifaceted impact on aquaculture

TABLE 1

Temperature impacts across biological systems of aquaculture organisms in aquaculture

Biological process	Temperature-Linked effects	Examples / Studies
Aerobic Capacity	Increased temperature raises oxygen demand while reducing oxygen solubility, potentially leading to hypoxia.	*Salmo salar* (Remen *et al.*, 2013); *Carassius auratus* (Fry and Hart, 1948);
Reproduction	Disrupted steroidogenesis, decreased fecundity, skewed sex ratios and altered timing.	*Salmo salar* (Pankhurst *et al.*, 2011); *Heterobranchus longifilis* (Legendre, 1986); *Dicentrarchus labrax* (*Sfakianakis et al.*, 2013)
Growth & Development	May be enhanced within optimal ranges but impaired outside of them; potential for compensatory growth.	*Dicentrarchus labrax, H. mollis, Lates calcarifer* (Zamora and Jeffs, 2012; Glencross and Bermudes, 2012)
Maturation	Can be accelerated or delayed; early maturation may be economically disadvantageous in salmonids.	*Conger myriaster* (Utoh *et al.*, 2013); *Salmo salar* (McClure *et al.*, 2007)
Phenology and Range Shift	Earlier seasonal events, poleward species shifts (100 km/decade) and altered bloom timing.	*Engraulis encrasicolus* (Beare *et al.*, 2004); *Trisopterus luscus* (Perry *et al.*, 2005); *Entelurus aequoreus* (Harris *et al.*, 2007)
Feed Conversion	Increased basal metabolic rate raises feed demand; FCR worsens at higher-than-optimal temperatures.	*Polyprion oxygeneios* (Khan *et al.*, 2014); *Argyrosomus mollis* (Zamora and Jeffs, 2012); *Lates calcarifer* (Glencross and Bermudes, 2012)
Nutrient Digestibility	Minimal direct effects unless beyond species-specific thermal limits; gut transit times may change.	*Seriola lalandi, Haliotis laevigata* (Miegel *et al.*, 2010; Currie *et al.*, 2015)

Temperature fluctuations in chronic way can work as stressors and further disrupt the delicate hormonal balance within these species, impacting neuro-endocrine glands. Osmo-regulatory mal functions and changed cardio-respiratory activities and immune responses are some notable physiological disruptions due to thermal stressors that imposes the fish species towards extinction (Paukert *et al.*, 2016; Stewart *et al.*, 2019 and Zhang *et al.*, 2019). Thus inhabitants of temperate zones including salmonids like *Salmo salar* and cod (*Gadus morhua*) are at risk, with higher mortality rates looming on the horizon (Abisha *et al.*, 2022). Temperature change impacts are already being reported from aquaculture localities like Vietnam (Binh *et al.,* 2017), Hawaii (McCoy *et al.,* 2017) and Australia (Hobday *et al.,* 2018). In the summer of 2004, Fraser River salmon in Canada suffered exceptionally high mortality as river temperatures rose to about 21.5 °C at Hell's Gate, surpassing the 60-year historical maximum recorded for that site. These unprecedented thermal conditions caused severe physiological stress, increased disease outbreaks and disrupted migration, leading to extensive en route mortality across multiple salmon runs (Brander, 2007). Interestingly, apart from warm-water species that face the heat; even cold-water inhabitants along with intertidal shellfish, find themselves in jeopardize situations as global temperatures are expected to rise by an average of 1.5 °C in this century (Hamdan *et al.*, 2012 and Gubbins *et al.*, 2013). For example, According to Pörtner *et al.* (2001), because of elevated temperature, two cold-water species Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*) exhibited reduced growth and reproductive performance. Additionally global warming poses a serious threat to cold-water aquaculture by shrinking suitable thermal habitats in freshwater ecosystems. Mohseni *et al.* (2003), predicted a 36 per cent decline in viable habitat for cold-water species and a 15 per cent reduction for cool-water species. In contrast, warm-water species could see a 31 per cent increase in suitable habitat. These projections were based on temperature tolerance data for 57 fish species across 764 stream sites in the United States. Changes in temperature and other environmental factors are affecting cold-water aquaculture industry in the Himalayan regions too, because of lower fish survival rates (Singh, 2015).

Inland water bodies, too, are facing the consequences of climate-induced temperature shifts. Temperature alterations in these ecosystems are reason behind elevations of some stress factors like reduced oxygen levels, compromised water quality and alterations in the availability of natural food sources for aquatic species (Woodward *et al.*, 2010). River systems-critical for global aquaculture-are projected to warm by 0.8-2.2°C, particularly in the USA, China, Europe and Australia (van vli *et al.*, 2013), raising concerns for temperature-sensitive production systems. The consequences ripple through the entire ecosystem.

Coastal aquaculture, another vital component of global food production, is with standing its own set of challenges from higher ocean temperatures. Rising temperatures obviously linked to lower productivity that impose higher management costs and ultimately creating significant threats to the economic and social sustainability of aquaculture operations. Temperature fluctuations in deep oceanic regions destroy thermal stratification, affecting nutrient distribution within the water. This, in turn, can trigger generation of toxic algal blooms and change the structure of the plankton community, disrupting food availability for various species and causing ramifications for coastal aquaculture systems (Seggel *et al.*, 2016). Aquaculture, especially when it involves filter-feeding creatures like oysters and mussels, is in trouble because these animals can consume the toxins from the harmful algae, which will bio-magnify and can reach ultimately to humans. Projections indicate that increasing ocean temperatures may enhance the bioaccumulation of chemical pollutants-such as Dechloranes 602, 604 and tetrabromobisphenol A (TBBPA)-in marine bivalves (Maulvault *et al.*, 2018). Elevated temperatures also promote greater absorption of heavy metals and other toxic substances in farmed molluscs, thereby increasing their vulnerability to

disease outbreaks (Abisha *et al.*, 2022). This poses a substantial threat to aquaculture performed in coastal and brackish water by potentially impacting seafood supply (Maulvault *et al.*, 2018). Communities in tropical regimes, engaged in coral and giant clam farming face heightened risks, such as increased losses due to bleaching events driven by rising sea temperatures.

Even, climate change induced temperature change poses significant challenges to a multibillion-dollar industry; the ornamental fisheries sector (Abisha *et al.*, 2022). Campos *et al.* (2019) experimented the thermal sensitivity of three ornamental fish species native to Amazonian forest streams and projected that climate change could lead to species-specific metabolic disruptions, ultimately impairing their physiological functions. Donelson *et al.* (2012), experimented on tropical reef fish and concluded that even slight increases in water temperature made them vulnerable. Since coral reefs serve as habitats for wild ornamental fish, climate change-induced reef degradation directly impacts the ornamental fish survival (Abisha *et al.*, 2022).

Ocean Acidification Threatens Aquatic Ecosystems and Aquaculture : Ocean acidification-a major outcome of climate changes-results from the sustained decline in oceanic pH caused by the uptake of CO, available at atmosphere (Richards *et al.*, 2015). As oceans absorb increasing amounts of CO_2, particularly under global warming scenarios exceeding 1.5°C, the adverse impacts on aquaculture are expected to intensify (Clements & Chopin, 2017 and IPCC, 2018). These changes threaten the stability and productivity of aquaculture operations, potentially leading to reduced yields and economic losses. Acidified waters hinder the development of calcium carbonate shells in calcifying organisms like shrimp, mussels, corals and oysters, which are essential organisms of marine aquaculture systems (Table 2). For example, reductions in wild oyster spat due to compromised coral skeleton development can disrupt the supply chain, compelling producers to depend more heavily on hatchery systems, thereby increasing operational costs and lowering profit margins (Blanchard *et al.,* 2017). Thus, in the U.S., 16 out of 23 coastal regions-where shellfish industries are economically vital-are considered highly vulnerable (Ekstrom *et al.*, 2015). Washington State's shellfish industry, for instance, has already experienced acidification-related losses, threatening livelihoods and a sector contributing over $270 million to the economy (NOAA, 2011 and WSBR Panel, 2012).

Table 2

Overview of ocean acidification impacts on shellfish developmental phases

Life Stage	Impacts	Species/Examples	References
Larval	Impaired calcification, shell dissolution, reduced metam orphosis, latent fitness effects	*Crassostrea gigas, C. virginica, Saccostrea glomerata, Mytilus edulis, A. irradians,* gastropods, crustaceans, echinoderms	Waldbusser *et al.*, 2010; Parker *et al.,* 2010; Gobler and Talmage, 2013
Juvenile	Size-dependent mortality, reduced growth, species specific tolerance	*Mercenaria mercenaria, Rudita - pes decussatus*	Waldbusser *et al.,* 2010
Adult	Reduced shell growth, weakened byssus threads, potential dwarfing	*Mytilid mussels, Nassarius corniculus*	O'Donnell *et al.*, 2013 and Garilli *et al.*, 2015
Seed Collection	Low larval survival threatens wild seed availability	Wild spat populations	Waldbusser *et al.*, 2015
Hatchery Phase	Need for carbonate chemistry control to ensure survival	Molluscan hatcheries	Barton *et al.*, 2012 and Ekstrom *et al.*, 2015

Ocean acidification significantly affects the physiological and metabolic functions of aquatic organisms by disrupting intercellular transport processes (Pörtner *et al.*, 2004). In acidified environments, the imbalance in acid-base regulation leads to oxidative stress, imposing additional energetic demands on the organism (Crocker and Cech, 1998). This stress is compounded by a decline in ATP synthesis and an increase in energy expenditure linked to intracellular acidosis, which can cause cellular damage, including harm to DNA, proteins and lipid membranes (Soriano-Santiago *et al.*, 2013).

Elevated atmospheric CO, poses a significant threat to the survival of anadromous species such as salmon, which rely heavily on their olfactory cues to locate natal streams for successful spawning (Ou *et al.*, 2015). For instance, Coho salmon have demonstrated notable behavioural disruptions and diminished olfactory sensitivity under high CO, conditions, highlighting their vulnerability to ocean acidification (Williams *et al.*, 2019).

Sea Level Rise and Its Threats to Sustainable Coastal Aquaculture Practices : Elevation in sea level due to melting land ice and the thermal dilation of oceans is another consequence of climate change that is predicted to pose significant challenges to marine coastal environments and in turn, the resilience of aquaculture practices.

Coastal ecosystems, such as mangroves and salt marshes and other intertidal zones, play important roles in sustaining fish fauna and providing seed stock for coastal as well as brackish-water aquaculture (Kibria *et al.*, 2017). But alterations in their habitats can result in shifts in species composition, abundance, distribution (Handisyde *et al.*, 2006 and Kibria *et al.*, 2017). Additionally, aquaculture production systems, including ponds, cages, tanks and pens, particularly in low-lying regions susceptible to saline water intrusion due to sea level rise (Kibria *et al.*, 2017). Salinity intrusion has already been observed by some striped catfish (*Pangasianodon hypophthalmus*) farmers in Vietnam (Nguyen *et al.*, 2018). Shrimp farming, in particular, is at risk due to its low-lying locations that lack protection from tidal surges, increasing their vulnerability to coastal flooding (Ahmed and Diana, 2015a). Thus near shore communities, who are dependent on aquaculture for their livelihoods, make them particularly vulnerable to the effects of aquatic salinization and declining fish habitats as a result of sea level rise (Dasgupta *et al.*, 2017).

Impact of Water Salinity Variations on Aquaculture : Salinity, a critical parameter in aquatic ecosystems, is subjected to fluctuations driven by climate change-induced factors like changes in precipitation levels, increased water loss through evaporation, accelerated ice melting, shifts in oceanic circulation and the blending of seawater with groundwater or subterranean flows (Koblinsky *et al.*, 2003 and Cochrane *et al.*, 2009). As the survivability of aquatic organisms is particularly linked to specific salinity levels, deviations from these thresholds pose a significant threat leading to reduced survival rates, compromised growth and impacting economic sustainability by increasing species losses and management costs. (Jahan *et al.*, 2019). For instance, exposure to salinity levels exceeding the physiological tolerance of striped catfish has been shown to reduce survival and growth performance, while negatively affecting blood cell profiles-implying a compromised immune response (Jahan *et al.*, 2019). However, it is essential to consider that the negative effect of elevated salinity levels appears to be more pronounced in aquaculture systems located in the lower reaches of coastal zones (Nguyen *et al.*, 2018). For instance, Ahmed, (2013) reported severe impacts on freshwater prawn production under elevated salinity conditions. Similarly, Baker *et al.* (2005) observed elevated death rate among clams when they are in juvenile stage, while Rodrick (2008), noted an enhanced likelihood of bacterial infection in oysters exposed to lower salinity environment.

Furthermore, variations in salinity have been linked to the immune responses of oysters, compromising the ability of blood cells to combat invading bacteria (Rodrick, 2008). While clams are known to possess wider tolerance level for salinity compared to other marine molluscs, they still cannot resist lengthen

Contact with highly elevated or drastically reduced salinity levels (Baker *et al.*, 2013). In coastal regions, fluctuations in salinity levels within a specific range have been associated with deadly diseases like white spot syndrome in shrimp farming (Liu *et al.*, 2006). In countries like Bangladesh, where income generation relies significantly on coastal aquaculture and fishing activities, the devastating effects of climate change, including rising aquatic salinity, have led to the destruction of fish habitats and economic hardship for local populations (Dasgupta *et al.*, 2017).

In addition, it is essential to understand that variations in salinity can also affect pathogen survival (Oidtmann *et al.*, 2017). Elevated salinity levels have been found to enhance the proliferation and persistence of specific pathogens, such as *Paramoeba perurans* together with ectoparasites like sea lice (Collins *et al.*, 2019). Additionally, the oyster parasite *Bonamia* sp. has been shown to thrive in higher salinity conditions (Arzul *et al.*, 2009). Consequently, drier and hotter summers, which may lead to increased salinity, could potentially elevate the population size and infectiousness of these parasites in aquatic environments. In contrast, reduced salinity during winter and spring might mitigate the enhanced survival of pathogens associated with warmer temperatures. Meanwhile, fungi and oomycetes are generally more prevalent in freshwater habitats (Van West, 2006). These interactions further underscore the complexities of salinity changes in aquatic ecosystems and their consequences for aquaculture.

Impact of Severe Climatic Events on Aquaculture : Extreme climatic incidents, including cyclones, waves and storms, are responsible in making the future of aquaculture development uncertain, particularly in seaside areas (Toussaint *et al.*, 2018). Sub-tropical areas are expected to face severe threats, such as the potential damage to production assets and livestock caused by rough sea conditions linked to more intense cyclones (Hamdan *et al.*, 2012).

Storm surges, intense waves and coastal erosion are considered the most threatening climatic hazards to aquaculture and related coastal industries. (Hamdan *et al.*, 2012). When severe storms strike, they frequently cause significant damage for aquaculture practitioners, primarily due to extensive damage to farms, thereby escalating the costs of rehabilitation (Maulu *et al.*, 2021). For instance, cage aquaculture is especially susceptible to the adverse impacts of cyclones and waves, as they can inflict substantial harm on the infrastructure employed in these operations. As a result there is a considerable risk for aquatic organisms to escapes due to equipment malfunctions. Cyclone Aila in 2009 devastated approximately 80 per cent of shrimp farms in the affected regions of Bangladesh (Kais and Islam, 2018). Similarly, storm events in Mexico in 2010 caused the destruction of around 1,200 farms, leading to an 80 per cent decline in the nation's annual tilapia production (Reid and Jackson, 2014). This, in turn, may necessitate site relocation or modifications in production methods, potentially exerting profound social and economic repercussions on aquaculture sustainability within these regions (Gubbins *et al.*, 2013).

Altered Precipitation Patterns on Aquaculture Sustainability : Changes in precipitation patterns have profound implications in aquaculture, in opposing ways. Whereas increased rainfall leads to flooding, extended periods of reduced rainfall causes drought. In this context, The Intergovernmental Panel on Climate Change (IPCC) notes that in certain regions, drought-related risks are projected to increase more sharply at 2 °C of global warming than at 1.5 °C (IPCC, 2018).

Heavy rainfall events are vulnerable to aquaculture, particularly in lowland areas. Risks encompass fish losses during floods, invasions of unwanted species, degradations of water quality and structural damage to aquaculture infrastructure, including wall erosion and infilling (Fig. 3) (Bell *et al.*, 2010). These incidents put at risk the economic and social dimensions of aquaculture sustainability, lowering producer's incomes and potentially driving increasing economic hardship in dependent populations. Furthermore, because of increased precipitation

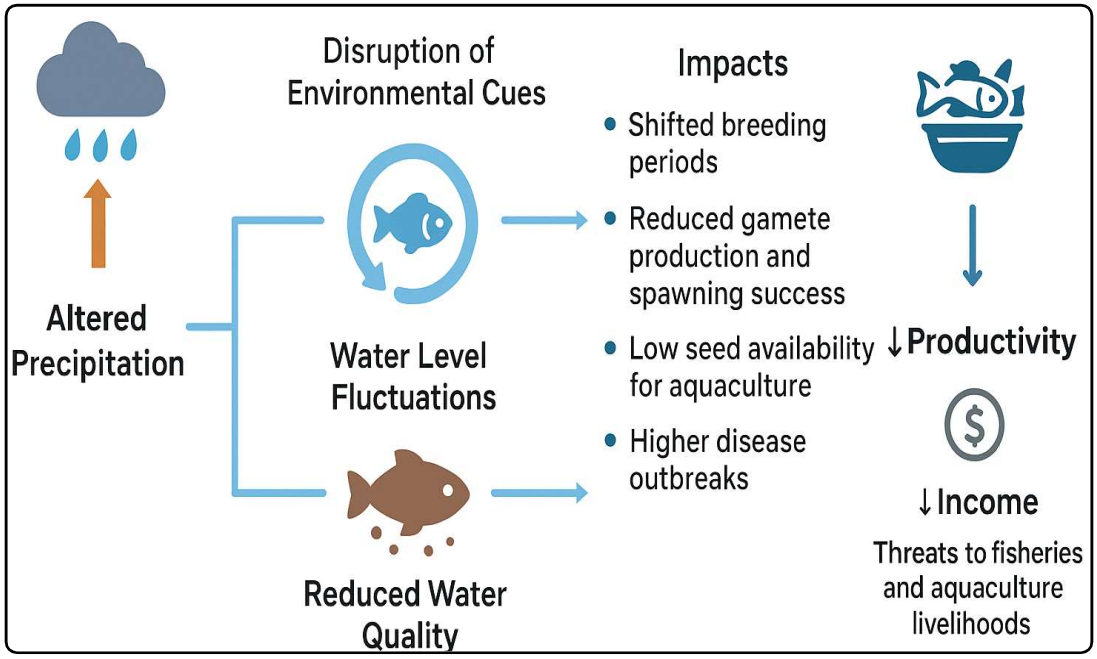

Fig. 3 : Conceptual framework illustrating how altered precipitation patterns due to climate change disrupt normal breeding of aquatic species

patterns and climatic events like storms and cyclones, coastal aquaculture faces unique challenges like saltwater intrusion in aquaculture ponds due to floods. For instance, tropical cyclones, common in South Asian countries, have intensified due to climate change, posing severe risks to aquaculture in low-lying areas (Knutson *et al.*, 2010).

Conversely, drought events resulting from elevated evaporation, lowered or erratic rainfall can induce water stress within aquaculture systems, leading to water shortages and declining water quality. Thus drought compromises water availability for aquaculture practices, leading to shortened culture periods, overcrowding of fish, higher waste metabolite levels, increased stress due to water scarcity and ultimately adversely impacting system output (Fig. 3) (Ahmed and Diana, 2015). The rising drought incidents in Australia have depleted freshwater resources, so fish farms must use poor-quality groundwater sources according to Gerlach *et al*., 2018. Furthermore, global warming is anticipated to bring in more frequent and lengthen droughts, affecting as many as 25 per cent of the globe's land area by 2050 (Park *et al.*, 2018) required for installation of new aquaculture settings.

These water-related challenges encompass economic, social and environmental aspects, collectively impacting aquaculture sustainability (Handisyde *et al.*, 2006; Barange *et al.*, 2018).

Changing climate on Aquatic Diseases and Harmful Algal Blooms : Climatic alterations across geographic locations have the capability to considerably elevate the risk of outbreak of diseases (Marcogliese, 2008 and Collins *et al.*, 2020). Because environmental changes can be a reason in promoting increased replication speed, enhanced virulence, extended life cycle duration and greater pathogen transmissibility like bacterial, parasitic, viral and/or fungal agents (Chiaramonte *et al.*, 2016 and Collins *et al.*, 2020). Climate change is contributing to the northward spread of protozoan parasites that threaten aquaculture in diverse regions. Two notable pathogens, *Perkinsus marinus* and *Haplosporidium nelsoni*, have expanded their ranges from the Gulf of Mexico to regions as far north as Delaware Bay and beyond, leading to widespread mortality in Eastern oysters (*Crassostrea virginica*). As because, cold conditions become less frequent, the continued poleward spread of this and similar pathogens is likely (Hofmann

et al., 2001). A striking example occurred in 1991 when *P. marinus* extended its northern limit from Chesapeake Bay to Maine-a 500/ km shift-in response to unusually mild winter conditions (Ford, 1996). Shifts in environmental conditions may also generate selective forces for instance thermal stress conditions, leading to the emergence of new and resistant pathogen strains with higher virulence (Murray & Peeler, 2005 and Panzarin *et al.*, 2014). For example, sea lice, notable warm water pathogens are expected to emerge as a considering factors in salmon culture, necessitating increased treatments related expenses (Collins *et al.*, 2020). Similarly, heightened temperature pressures may lead to rise of more epizootic diseases, posing significant economic challenges and influencing the economic and societal dimensions that underpin sustainable aquaculture (Sae-Lim *et al.*, 2017 and Maulu *et al.*, 2019). Several case-specific examples further illustrate the detrimental effects of temperature increases on aquaculture species:

Atlantic Salmon (Salmo salar) : In tank-based experiments, rising temperatures have been shown to accelerate the development of infective stages of the salmon louse (*Lepeophtheirus salmonis*), a parasitic copepod that significantly impacts salmon aquaculture (Hamre *et al.,* 2019).

American Lobster (*Homarus americanus*) : In Massachusetts, USA, warming waters have been linked to epizootic shell disease, characterized by microbial dysbiosis, shell erosion and increased mortality. This condition undermines shell integrity and overall lobster health (Groner *et al.*, 2018 and Ishaq *et al.*, 2023).

Shrimp Species (e.g., *Penaeus vannamei*)

Across regions like Mexico, Thailand and the USA, outbreaks of Acute Hepatopancreatic Necrosis Disease (AHPND) - also known as early mortality syndrome - have been associated with toxigenic strains of *Vibrio sp.*, particularly *V. parahaemolyticus*. Elevated temperatures and salinity levels modulate the expression of the *PirA* gene responsible for toxin production (Estrada-Perez *et al.*, 2020 and Lee *et al.*, 2015). Thus, for instance, increased outbreaks of *Vibrio*-related mortalities have been reported in shrimp farms along the U.S. Gulf Coast, according to Mayer *et al.*, 2014. Similarly, in the Mediterranean region, *Vibrio* infections in aquaculture facilities have risen in correlation with elevated sea surface temperatures (Bostancż *et al.*, 2015).

Shrimp (*Penaeus setiferus* and *Penaeus aztecus*)

On the Texas Gulf Coast, black gill disease, caused by the ciliate *Hyalophysa lynni*, has been linked to high temperatures (>20°C) and reduced salinity (<10 ppt) following heavy precipitation (Swinford *et al.*, 2021).

Blue Crab (*Callinectes sapidus*)

Infections by the dinoflagellate *Hematodinium perezi* have become more prevalent along the U.S. Atlantic seaboard, with warmer waters (>25°C) and high salinity (>30 ppt) supporting parasite development. Additionally, milder winters have enhanced parasite survival rates (Shields, 2019).

Furthermore, emergence of harmful algal blooms (HABs) because of climate alterations in aquatic environments pose another considerable threat (Wasmund *et al.*, 1998; Edwards *et al.*, 2006; Moore *et al.*, 2008 and Trainer *et al.*, 2020). Taxonomic groups like flagellates and dinoflagellates, with other potential harmful toxic species, have been reported for elevating stress or higher mortality in shellfish and finfish (Gubbins *et al.*, 2013 and Basti *et al.*, 2011). For example, aquaculture in the country Chile suffered from higher fish mortality rates because of the occurrence of *Alexandrium catenella* and *Pseudochattonella verruculosa*, outbreaks of whose were linked to variations in thermal layering of the water column caused by climate dynamics (Trainer *et al.*, 2020). Additionally, several research works have showcased that HABs are linked to disease symptoms such as atrophy, inflammation and necrosis in bivalve molluscs (Haberkorn *et al.*, 2010; Basti *et al.*, 2011 and Hégaret *et al.*, 2012). Unfortunately, understanding of the pathways linking climate change to toxic substance

accumulation in aquaculture is still developing and remains incomplete (Farrell *et al*., 2015).

Mitigation Strategies

In recent times, aquaculture comes out as a potential alternative to fulfil the enlarged demand of food across globe. Therefore, it is become relevant to correctly point out and address the threats for the industry across different geographical regions, climatic conditions and production systems in relation to climate change. Mitigation as well as adaptation strategies are the core parts to counter the difficulties arising from climate change in aquaculture to maintain the sector's adaptability and long-term viability amid changing environmental circumstances. Though adapting to these changes is a multifaceted approach, aquaculture producers must embrace available adaptation options, such as diversification of species and systems like selective breeding of aquaculture species for traits like disease resistance and adaptability to shifting environmental conditions as well as the expansion of farming into more diverse environments can bolster resilience and mitigate immediate risks. Diversification in livelihoods by combining aquaculture with other sectors, such as agriculture, can provide multiple income sources and enhance resilience (Maulu *et al.*, 2021). Beside, incorporating indigenous knowledge alongside scientific approaches can improve adaptation strategies by incorporating specific insights about local environments, weather patterns and resource management so that aquaculture operations can be easily adapted in changing climate conditions. Aquaculture producers can reduce their carbon emission amount by imposing sustainable feed production practices and efficient wastewater management (Maulu *et al.*, 2021). Additionally, application of sinking feeds, feed types with lower environmental impacts can be a pathway to reduce emissions associated with aquaculture (Maulu *et al.*, 2021). Transition from non-renewable to renewable energy sources, particularly solar energy, for aquaculture operations is another effective means to mitigate the industry's carbon footprint. In addition, community-based adaptations, like the development of flood control structures and embedding climate concerns into economic planning and management are vital steps toward rescuing the future of aquaculture from changing climate.

This review has offered significant insights in highlighting the critical intersection of climate change and aquaculture production from rising temperatures and acidification to salinity shifts and extreme weather events. However, it reveals critical gaps in data, localized understanding and adaptive frameworks. Most of the cited literature offers generalized insights or isolated case studies, often lacking longitudinal datasets or region-specific projections. Despite the emphasis on physiological impacts, relatively few studies integrate ecological, economic and social dimensions, leaving a fragmented picture of climate vulnerability across aquaculture systems.

Moreover, although adaptation strategies like selective breeding, system diversification and renewable energy adoption are frequently recommended, their real-world feasibility, economic viability and long-term sustainability are underexplored. Indigenous knowledge, while acknowledged, is often side-lined in favour of technical solutions, indicating a disconnect between global policy recommendations and community-level implementation.

To truly fortify aquaculture against climate-driven disruptions, research must move beyond descriptive accounts and into predictive modelling, cross-disciplinary risk assessments and scalable pilot interventions. Emphasis should be placed on Developing robust early-warning systems for disease outbreaks and harmful algal blooms, Integrating ecological restoration (e.g., mangrove rehabilitation) with aquaculture zoning, Encouraging participatory policy frameworks that involve local stakeholders in climate governance, Expanding investment in socio-ecological resilience-including insurance models, climate-smart aquaculture certification and inclusive technology transfer.

In absence of decisive and regionally tailored interventions, the trajectory of aquaculture in a warming world risks becoming increasingly unstable. Future research studies should target to offer a more thorough understanding of the intricate relationship between climate change and aquaculture, as well as to develop and refine effective adaptation strategies. Disastrous effects of changed climate on aquaculture if not address immediately; the challenges arise because of climate change will only intensify, making it hard to maintain sustainable production in aquaculture and culture-based fisheries for upcoming decades.

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Assessment of Planting Density and Nutrient Levels on Macronutrient Uptake in Broccoli (*Brassica oleracea* var. *italica*) Hybrids and Residual Soil Nutrient Status under Open Field Conditions

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Abstract

An experiment was carried out to evaluate the effects of varying nutrient levels and spacings on soil nutrient status and nutrient uptake by broccoli hybrids under open field condition at *Sanjeevini Vatika*, Department of Horticulture, UAS, GKVK, Bengaluru, Karnataka, during the *Rabi* season of 2023. The study was designed as a factorial Randomized Complete Block Design (RCBD) with three replications, encompassing three factors. Among the treatments, T_8 ($H_2S_2N_2$), which involved the NS1250 hybrid planted at wider spacing combined with higher NPK application, exhibited significantly enhanced uptake of nitrogen, phosphorus and potassium. The treatment T_8 recorded maximum uptake of nitrogen (225.47 kg ha^{-1}), phosphorus (60.99 kg ha^{-1}) and potassium (175.69 kg ha^{-1}) by the plant.TreatmentT_5 ($H_2S_1N_1$) involving the same hybrid planted at closer spacing and receiving a lower nutrient dosage recorded the higher nutrient use efficiency: 56.38 kg per kg for nitrogen, 38.64 kg per kg for phosphorus and 90.51 kg per kg for potassium.The treatment T_8 ($H_2S_2N_2$) proved most effective for maximizing nutrient uptakeas it allowed plants to access sufficient space and nutrients, resulting in significantly higher absorption of nitrogen, phosphorus and potassium compared to other treatments.

Keywords : Broccoli, Spacing, Nitrogen, Phosphorus, Potassium, Uptake

Broccoli (*Brassica oleracea* var *italica*), a healthy, nutritious and exotic vegetable belonging to family crucifer. The name 'broccoli' originates from the Latin word *'brachium'*, meaning 'arm' or 'branch'. The compact heads are encircled by numerous leaves, which constitute the largest portion of the mature plant. Historically, broccoli is believed to have evolved from a wild cabbage species native to the coastal regions of Italy and Greece (Nagraj *et al.,* 2020). In India, broccoli cultivation is largely confined to temperate regions such as Himachal Pradesh, Uttarakhand, the Nilgiri Hills and selective areas of Maharashtra and Karnataka, where productivity remains relatively low.

The floral biology of broccoli resembles to that of other cole crops, featuring 4 petals and sepals with 6 stamens and 2 carpels (Kumar, 2021). For every 100 g of broccoli edible portion provides 6.64 g of carbohydrates, 2.81 g of protein, 1.6 g of sugars and supplies 34 kcal energy. It also contains vitamin A (4%), 89.31 g of water along with essential minerals including 47 mg of calcium and 316 mg of potassium, (Pankaj *et al.,* 2018).

Plant nutrients are fundamental to promoting vigorous plant growth, ensuring optimal development and achieving superior-quality yields. Broccoli, being a high nitrogen-demanding crop, requires adequate nutrient input to support vigorous growth and the formation of healthy, marketable heads as nitrogen being an essential component of proteins via amino acids, chlorophyll, nucleotides, alkaloids and enzymes. Nitrogen is essential for photosynthesis, vigorous vegetative growth and dry matter production which is very essential for growth and development of broccoli (Aswini *et al.,* 2024). Phosphorous promotes better root development, flowering and fruit production in addition to aiding in the synthesis of nucleic acids like DNA and RNA. Potassium plays a crucial role in nutrient transport and the regulation of stomatal opening and closing. A deficiency of potassium reduces the plant's ability to absorb photosynthetic carbon, leading to slower growth and decreased yield (Reddy *et al.*, 2024). Hence, precise and balanced nutrient management is essential to maintain soil fertility and optimize crop performance. However, in India, broccoli cultivation lacks a well-defined package of practices, resulting in the adoption of suboptimal nutrient regimes and poorly managed agronomic practices that constrain yield potential. To overcome these challenges, focused research and strategic agronomic interventions are imperative for enhancing productivity and ensuring sustainable cultivation. Therefore, the present investigation was undertaken to study the influence of plant spacing and nutrient levels on soil nutrient status and nutrient uptake by broccoli hybrids.

Material and Methods

An experiment was conducted in the *Rabi* season of 2023 at *Sanjivani Vatika*, Department of Horticulture, University of Agricultural Sciences, GKVK, Bengaluru. The experiment was arranged in a factorial randomized complete block design with three replications, considering plant hybrids as first factor (3 hybrids: H_1-Lucky, H_2-NS1250 and H_3-Saki), plant spacing as the second factor (2 levels: S_1-45 cm × 45 cm & S_2-60 cm ×45 cm) and major nutrients as the third factor (2 levels: N_1-120:80:60 kg NPK ha^{-1} & N_2-150:100:75 kg NPK ha^{-1}). Fifty per cent dose of nitrogen, along with the full doses of phosphorus and potash were applied as basal fertilizers. The remaining 50 per cent of nitrogen was top dressed 30 days after transplanting in each plot, according to the treatments. The experimental field was well-ploughed prior to transplanting and soil samples were collected before planting to analyze the chemical properties and the nutrient status. Analysis revealed slightly acidic soil pH (5.84) and normal electrical conductivity (EC) of 0.28 dS m^{-1}. Organic carbon was found low (0.41%), both available nitrogen and potassium were found low (169.28 kg ha^{-1} and 157.49 kg ha^{-1}), while available phosphorus found medium (47.68 kg ha^{-1}). The soil is deficient in zinc (0.39 mg kg^{-1}) and boron (0.26 mg kg^{-1}) content whereas, sufficient in iron (4.31 mg kg^{-1}), manganese (3.56 mg kg^{-1}) and copper (3.33 mg kg^{-1}) content. After crop harvest from each plot, a composite soil samples were collected from the 0-30 cm depth. The soil samples were chemically analyzed by following the standard procedures. Soil pH and electrical conductivity (EC) were measured using a digital meter as described by Jackson (1973), while soil organic carbon was determined by the Walkley and Black method (1934). Available nitrogen, phosphorus and potassium contents in the soil were analyzed according to the methods outlined by Subbaiah & Asija (1956), Bray & Kurtz (1945) and Jackson (1973), respectively. Available micronutrients were analyzed following the method described by Lindsay and Norwell (1978) and available boron was determined by following the method described by John *et al.* (1975).

Plant nutrient uptake was analyzed using various methodologies: nitrogen content was measured by the micro-Kjeldahl method, phosphorus content by theVanado-molybdo phosphoric acid yellow color method as described by Jackson (1973) and potassium content by the same method used for available potassium in soil analysis. The recorded data for all the parameters were statistically analyzed using OPSTAT software.

Results and Discussion

Soil Nutrient Status after Harvest and the Plant Nutrient Uptake of Broccoli

Following the broccoli harvest, measurements of soil pH, electrical conductivity (EC) and organic carbon content showed no significant differences across the various plant spacing treatments, nutrient application rates and their combinations in open field condition. Comprehensive data on these soil properties are presented in Table 1.

The interaction effect of hybrids, plant spacing and nutrient levels on post-harvest soil availability of nitrogen, phosphorus and potassium and on nutrient uptake by broccoli hybrids was found significant and the data is presented in Table 2.

Impact of hybrids: Among the evaluated hybrids, H_2 (NS1250) recorded lower residual soil available nitrogen with values of 88.27 kg ha^{-1}, lower level of available phosphorus (35.28 kg ha^{-1}) and available potassium (128.48 kg ha^{-1}). In comparison, the hybrid H_1 (Lucky) recorded comparatively higher available nitrogen, phosphorus and potassium levels after harvest. Hybrid NS1250 (H_2) absorbed significantly higher amounts of nitrogen (203.99 kg ha^{-1}), phosphorus (55.66 kg ha^{-1}) and potassium (163.61 kg ha^{-1}) compared to the other hybrids.

Table 1

Effect of hybrids, plant spacing and nutrient levels on pH, EC and OC of soil at harvest under open field condition

Treatment	pH	Electrical conductivity (dS m^{-1})	Organic carbon (%)
T_1 - $H_1S_1N_1$	6.13	0.23	0.43
T_2 - $H_1S_1N_2$	6.13	0.22	0.43
T_3 - $H_1S_2N_1$	6.14	0.25	0.43
T_4 - $H_1S_2N_2$	6.12	0.24	0.43
T_5 - $H_2S_1N_1$	6.15	0.24	0.42
T_6 - $H_2S_1N_2$	6.14	0.24	0.43
T_7 - $H_2S_2N_1$	6.13	0.23	0.43
T_8 - $H_2S_2N_2$	6.17	0.25	0.45
T_9 - $H_3S_1N_1$	6.16	0.24	0.43
T_{10} - $H_3S_1N_2$	6.13	0.23	0.43
T_{11} - $H_3S_2N_1$	6.12	0.22	0.42
T_{12} - $H_3S_2N_2$	6.14	0.23	0.42
F-test	NS	NS	NS
S.Em±	0.03	0.01	0.01
CD (5%)	-	-	-

*Significance @ 5% level, NS – Non significant

H_1: Lucky S_1: 45 cm × 45 cm N_1: 120:80:60 kg NPK ha^{-1}
H_2: NS1250 S_2: 60 cm × 45 cm N_2: 150:100:75 kg NPK ha^{-1}
H_3: Saki

Table 2

Effect of hybrids, plant spacing and nutrient levels on available nutrients in the soil at harvest (nitrogen, phosphorus and potassium) and on nutrient uptake by broccoli hybrids

Treatments	Available nutrients in the soil at harvest (kg ha^{-1})			Nutrient uptake by broccoli hybrids (kg ha^{-1})		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Hybrids (H)						
H_1	98.83	42.02	139.72	162.75	39.24	131.13
H_2	88.27	35.28	128.48	203.99	55.66	163.61
H_3	91.43	36.86	131.39	186.16	51.17	154.22
F-test	*	*	*	*	*	*
S.Em±	0.40	0.25	0.40	1.07	0.44	0.88
CD (5%)	1.18	0.74	1.16	3.13	1.28	2.59

Continued....

Table 2 Continued....

Treatments	Available nutrients in the soil at harvest (kg ha^{-1})			Nutrient uptake by broccoli hybrids (kg ha^{-1})		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Plant spacings (S)						
S_1	91.22	37.46	132.25	169.71	42.82	137.55
S_2	94.46	38.64	134.13	198.89	54.56	161.75
F-test	*	*	*	*	*	*
S.Em±	0.33	0.21	0.32	0.87	0.36	0.72
CD (5%)	0.96	0.61	0.95	2.56	1.51	2.11
Nutrient levels (N)						
N_1	90.55	36.70	130.82	178.16	45.83	143.61
N_2	95.13	39.41	135.56	190.44	51.55	155.69
F-test	*	*	*	*	*	*
S.Em±	0.33	0.21	0.32	0.87	0.36	0.72
CD (5%)	0.96	0.61	0.95	2.56	1.04	2.11

*Significance @ 5% level, NS – Non significant

H_1: Lucky
H_2: NS1250
H_3: Saki

S_1: 45 cm × 45 cm
S_2: 60 cm × 45 cm

N_1: 120:80:60 kg NPK ha$^{-1,}$
N_2: 150:100:75 kg NPK ha^{-1}

Impact of spacing : Regarding plant spacing, the lower residual soil available nutrient was recorded in closer spacing (45 cm × 45 cm) with the values of 91.22 kg ha^{-1} of available nitrogen, 37.46 kg ha^{-1} of phosphorus and 132.25 kg ha^{-1} of potassium. Meanwhile, maximum available nutrients after harvest of broccoli were recorded in wider spacing (60 cm × 45 cm). The maximum uptake of nutrient was recorded in wider spacing of 60 cm × 45 cm with values of 198.89 kg ha^{-1} of nitrogen uptake, 54.56 kg ha^{-1} of phosphorus and 161.75 kg ha^{-1} of potassium.

Impact of nutrient : The higher level of nutrients dosage (N_2: 150:100:75 kg NPK ha^{-1}) recorded maximum residual available nitrogen (95.13 kg ha^{-1}), phosphorus (39.41 kg ha^{-1}) and potassium (135.56 kg ha^{-1}) compared to lower dose of nutrients. Also recorded maximum uptake of nutrient was recorded in N_2 with the values of 190.44 kg ha^{-1} nitrogen uptake, 51.55 kg ha^{-1} of phosphorus and 155.69 kg ha^{-1} of potassium.

The significant difference in nutrient uptake and residual soil nutrients among hybrids might be due to genetic variation in nutrient absorption efficiency, plant density effects on root growth and competition which may have affected the uptake of nutrients. The closer spacing depleted nutrients faster due to higher plant competition when compared to wider spacing, which has provided enough space for proper root growth and varied uptake. Higher nutrient doses generally increased both nutrient uptake by plants and residual soil nutrient levels, reflecting a balance between supply and plant demand. The findings are further supported by Abhijithnaik *et al.,* 2023 in broccoli.

Two-way Interactions on the Soil Nutrient Impact of Status at Harvest and Nutrient Uptake by Broccoli

The combined two-way interaction of broccoli hybrids, spacing dimensions and nutrient levels significantly influenced the residual availability of nitrogen, phosphorus and potassium in the soil after

TABLE 3

Interaction effect of hybrids, plant spacing and nutrient levels on soil nutrients availability at harvest (nitrogen, phosphorus and potassium) and on nutrient uptake by broccoli hybrids

Treatments	Available nutrients in the soil at harvest (kg ha^{-1})			Nutrient uptake by broccoli hybrids (kg ha^{-1})		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
(H×S)						
H_1S_1	97.48	41.13	138.49	150.55	32.60	120.04
H_1S_2	100.18	42.90	140.95	174.95	45.89	142.22
H_2S_1	85.28	34.40	126.79	185.03	50.78	152.60
H_2S_2	91.26	36.16	130.17	222.95	60.55	174.62
H_3S_1	90.91	36.86	131.48	173.55	45.09	140.02
H_3S_2	91.95	36.87	131.29	198.77	57.24	168.42
F-test	*	*	*	*	*	*
S.Em±	0.57	0.36	0.56	1.51	0.62	1.25
CD (5%)	1.67	1.05	1.65	4.43	1.81	3.66
(H×N)						
H_1N_1	98.08	41.55	139.21	158.52	37.11	126.58
H_1N_2	99.57	41.90	139.51	166.98	41.37	135.68
H_2N_1	84.89	32.62	124.47	198.12	53.05	159.49
H_2N_2	91.65	37.94	132.48	209.86	58.28	167.73
H_3N_1	88.68	35.34	128.07	177.84	47.32	144.77
H_3N_2	94.18	38.39	134.70	194.49	55.01	163.67
F-test	*	*	*	*	*	*
S.Em±	0.57	0.36	0.56	1.51	0.62	1.25
CD (5%)	1.67	1.05	1.65	4.43	1.81	3.66
(S×N)						
S_1N_1	89.47	36.67	130.81	161.44	38.72	129.83
S_1N_2	92.97	38.25	133.70	177.98	46.93	145.28
S_2N_1	91.63	36.72	130.84	194.88	52.94	157.40
S_2N_2	97.30	40.57	137.43	202.90	56.18	166.10
F-test	*	*	*	*	*	*
S.Em±	0.46	0.29	0.46	1.23	0.50	1.02
CD (5%)	1.36	0.86	1.34	3.61	1.48	2.99

*Significance @ 5% level, NS – Non significant

H_1: Lucky
H_2: NS1250
H_3: Saki

S_1: 45 cm × 45 cm
S_2: 60 cm × 45 cm

N_1: 120:80:60 kg NPK ha$^{-1,}$
N_2: 150:100:75 kg NPK ha^{-1}

crop harvest, as well as the nutrient uptake by broccoli hybrids. The corresponding data are presented in Table 3.

Combined impact of hybrids and plant spacings: Significantly higher available nitrogen, phosphorus and potassium in soil at harvest were observed in the treatment H_1S_2 (Lucky + 60 cm × 45 cm spacing), with available nitrogen values of 100.18 kg ha^{-1}, available phosphorus of 42.90 kg ha^{-1} and available potassium of 140.95 kg ha^{-1}. The lower available nitrogen was recorded in H_3S_1 (Saki + 45 cm × 45 cm spacing) with the values of 90.91 kg ha^{-1}, whereas H_2S_1 (NS1250 + 45 cm × 45 cm) exhibited lower available phosphorus (34.40 kg ha^{-1}) and potassium (126.79 kg ha^{-1}).

The maximum uptake of nutrients by broccoli was recorded (in the treatment) H_2S_2 (NS1250 + 60 cm × 45 cm) with uptake of 222.95 kg ha^{-1} of nitrogen, 60.55 kg ha^{-1} of phosphorus and 174.62 kg ha^{-1} of potassium. In contrast the lower uptake of nitrogen, phosphorus and potassium were recorded by treatment combination of H_1S_1 (Lucky+45 cm×45 cm) with the values of 150.55 kg ha^{-1} of nitrogen, 32.60 kg ha^{-1} of phosphorus and 120.04 kg ha^{-1} of potassium.

Combined impact of hybrids and nutrient levels: Among the various hybrid and nutrient combinations evaluated, the Lucky hybrid (H_1) when applied with 150:100:75 kg NPK per hectare (N_2) resulted in the higher residual levels of nutrients in soil after harvest, with available nitrogen, phosphorus and potassium of 99.57 kg ha^{-1}, 41.90 kg ha^{-1} and 139.51 kg ha^{-1}, respectively. Conversely, the NS1250 hybrid (H_2) when applied with 120:80:60 kg NPK per hectare (N_1) registered the lower nutrient availability for nitrogen (84.89 kg ha^{-1}), phosphorus (32.62 kg ha^{-1}) and potassium (124.47 kg ha^{-1}) in the soil at the harvest.

In terms of nutrient uptake, the combination of H_2N_2 (NS1250 + 150:100:75 kg NPK ha^{-1}) recorded the higher uptake of nitrogen, phosphorus and potassium, with values of 209.86 kg ha^{-1}, 58.28 kg ha^{-1} and 167.73 kg ha^{-1}, respectively. On the other hand, the combination of H_1N_1 (Lucky + 120:80:60 kg NPK ha^{-1}) demonstrated the lower uptake, registering 158.52 kg ha^{-1} of nitrogen, 37.11 kg ha^{-1} of phosphorus and 126.58 kg ha^{-1} of potassium.

Combined impact of plant spacing and nutrient levels: Among the different combinations of spacing and nutrient application assessed, the treatment comprising S_2N_2 (60 cm × 45 cm + 150:100:75 kg NPK ha^{-1}) recorded the higher availability of nutrients in soil with residual nitrogen, phosphorus and potassium content of 97.30 kg ha^{-1}, 40.57 kg ha^{-1} and 137.43 kg ha^{-1}, respectively. In contrast, the lower nutrient levels after harvest were observed under S_1N_1 (45 cm × 45 cm + 120:80:60 kg NPK ha^{-1}), with available nitrogen, phosphorus and potassium content of 89.47 kg ha^{-1}, 36.67 kg ha^{-1} and 130.81 kg ha^{-1}, respectively.

Regarding nutrient uptake, the combination of S_2N_2 (60 cm × 45 cm + 150:100:75 kg NPK ha^{-1}) recorded the maximum uptake of macronutrients with nitrogen, phosphorus and potassium uptake amounting to 202.90 kg ha^{-1}, 56.18 kg ha^{-1} and 166.10 kg ha^{-1}, respectively. Meanwhile, the lower uptake values were found in the S_1N_1 (45 cm × 45 cm + 120:80:60 kg NPK ha^{-1}) treatment with nitrogen, phosphorus and potassium uptake value of 161.44 kg ha^{-1}, 38.72 kg ha^{-1} and 129.83 kg ha^{-1}, respectively.

The combined effects of broccoli hybrids, spacing and nutrient levels on soil nutrient availability and broccoli uptake are influenced by the interaction of plant genetics, growing conditions and nutrient supply. Wider spacing likely reduces plant competition, resulting in higher residual soil nutrients, especially with the Lucky hybrid. The observed interactions indicate that the NS1250 hybrid coupled with wider spacing of 60 cm × 45 cm under higher nutrient levels enhances nutrient uptake and crop performance, reflecting its superior nutrient absorption efficiency and biomass production potential. These findings highlight that optimizing hybrid selection along with appropriate spacing and nutrient management enhances soil fertility conservation.

Three-way Interactions on the Soil Impact of Nutrient Status after Harvest and the Plant Nutrient Uptake of Broccoli

The available nitrogen, phosphorus and potassium in the soil after harvest, along with the uptake of

TABLE 4

Interaction effect of hybrids, plant spacing and nutrient levels on available nutrients in the soil at harvest (nitrogen, phosphorus and potassium) and on nutrient uptake by broccoli hybrids

Treatments	Available nutrients in the soil at harvest (kg ha^{-1})			Nutrient uptake by broccoli hybrids (kg ha^{-1})		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
(H×S×N)						
T_1 - $H_1S_1N_1$	98.16	42.69	140.38	148.62	31.25	118.59
T_2 - $H_1S_1N_2$	96.80	39.57	136.61	152.49	33.95	121.49
T_3 - $H_1S_2N_1$	98.01	41.57	139.47	168.42	42.98	134.57
T_4 - $H_1S_2N_2$	102.34	44.24	142.42	181.47	48.79	149.86
T_5 - $H_2S_1N_1$	81.42	31.45	122.86	175.81	45.99	145.43
T_6 - $H_2S_1N_2$	89.14	37.34	130.71	194.24	55.56	159.76
T_7 - $H_2S_2N_1$	88.37	33.79	126.08	220.43	60.11	173.55
T_8 - $H_2S_2N_2$	94.16	38.53	134.25	225.47	60.99	175.69
T_9 - $H_3S_1N_1$	88.85	35.88	129.19	159.88	38.91	125.46
T_{10} - $H_3S_1N_2$	92.97	37.83	133.77	187.22	51.27	154.57
T_{11} - $H_3S_2N_1$	88.51	34.79	126.95	195.79	55.73	164.08
T_{12} - $H_3S_2N_2$	95.39	38.94	135.62	201.75	58.76	172.76
F-test	*	*	*	*	*	*
S.Em±	0.80	0.51	0.79	2.13	0.87	1.77
CD (5%)	2.35	1.49	2.33	6.26	2.55	5.18

*Significance @ 5% level, NS – Non significant

H_1: Lucky
H_2: NS1250
H_3: Saki

S_1: 45 cm × 45 cm
S_2: 60 cm × 45 cm

N_1: 120:80:60 kg NPK ha^{-1}
N_2: 150:100:75 kg NPK ha^{-1},

macronutrients by the hybrids was significantly influenced by the three-way interaction of broccoli hybrids, plant spacing and nutrient levels. The results are summarized in Table 4.

The combined treatment $H_2S_1N_1$ (T_5: NS1250 + 45 cm × 45 cm + 120:80:60 kg NPK ha^{-1}) recorded the lower residual nutrient levels in soil after harvest with 81.42 kg ha^{-1} of nitrogen, 31.45 kg ha^{-1} of phosphorus and 122.86 kg ha^{-1} of potassium. In contrast, the higher available nutrient content in soil were observed under $H_1S_2N_2$ (T_4: Lucky + 60 cm × 45 cm + 150:100:75 kg NPK ha^{-1}), registering 102.34 kg ha^{-1} of nitrogen, 44.24 kg ha^{-1} of phosphorus and 142.42 kg ha^{-1} of potassium.

Nutrient uptake by broccoli plants varied significantly across treatments. The greater uptake of macro nutrients-nitrogen, phosphorus and potassium were recorded in the treatment $H_2S_2N_2$ (T_8: NS1250 + 60 cm × 45 cm + 150:100:75 kg NPK ha^{-1}), with respective values of 225.47 kg ha^{-1} for nitrogen, 60.99 kg ha^{-1} for phosphorus and 175.69 kg ha^{-1} for potassium. This treatment was *on par* with the combination treatment $H_2S_2N_1$ (T_7: NS1250 + 60 cm × 45 cm + 120:80:60 kg NPK ha^{-1}), which recorded nutrient uptake values of 220.43 kg ha^{-1} for nitrogen, 60.11 kg ha^{-1} for phosphorus and 173.55 kg ha^{-1} for potassium. Conversely, the lower uptake of these nutrients was observed under $H_1S_1N_1$ (T_1: Lucky + 45 cm × 45 cm + 120:80:60 kg NPK ha^{-1}), where

nitrogen, phosphorus and potassium were absorbed at levels of 148.62 kg ha^{-1}, 31.25 kg ha^{-1} and 118.59 kg ha^{-1}, respectively. The Lucky hybrid exhibits a slower growth rate compared to other hybrids, possibly due to its lower nutrient uptake efficiency, which may have resulted in more nutrients residual in the soil after harvest rather than being utilized by the crop. Wider spacing reduces competition among plants for nutrients, potentially leaving more nutrients available in the soil post-harvest. In contrast, denser spacing leads to higher nutrient uptake, likely due to a greater number of plants per unit area and increased competition for nutrients. A higher nutrient application rate increases the total nutrients supplied, which can cause higher residual nutrient concentrations, while a lower nutrient rate resulted in less nutrient input overall, contributing to lower residual nutrient levels. The interaction of hybrid vigor, plant spacing and nutrient application rates collectively influences nutrient uptake dynamics and residual soil nutrient status. Thus, a less nutrient-demanding hybrid grown at wider spacing with higher nutrient levels may leave more residual nutrients compared to a high-demand hybrid under closer spacing with lower fertilizer application. Similar findings were reported by Kumar *et al.* (2018), Yildirim *et al.* (2020), Hussain *et al.* (2021) and Abhijithnaik *et al*. (2023) in broccoli.

Table 5

Interaction effect of hybrids, plant spacing and nutrient levels on nutrient use efficiency

Treatment	NUE (kg kg^{-1})	PUE (kg kg^{-1})	KUE (kg kg^{-1})
T_1 - $H_1S_1N_1$	36.08	25.12	72.16
T_2 - $H_1S_1N_2$	37.13	26.70	74.27
T_3 - $H_1S_2N_1$	25.85	23.23	45.45
T_4 - $H_1S_2N_2$	26.44	23.52	48.74
T_5 - $H_2S_1N_1$	56.38	38.64	90.51
T_6 - $H_2S_1N_2$	45.47	33.12	89.08
T_7 - $H_2S_2N_1$	40.37	31.55	80.74
T_8 - $H_2S_2N_2$	39.38	30.07	78.77
T_9 - $H_3S_1N_1$	55.25	36.14	89.72
T_{10} - $H_3S_1N_2$	44.86	32.17	87.80
T_{11} - $H_3S_2N_1$	35.95	24.93	71.90
T_{12} - $H_3S_2N_2$	36.56	25.84	73.12

H_1: Lucky S_1: 45 cm × 45 cm N_1: 120:80:60 kg NPK ha^{-1}
H_2: NS1250 S_2: 60 cm × 45 cm N_2: 150:100:75 kg NPK ha^{-1};
H_3: Saki

Nutrient use Efficiency

The higher Nitrogen Use Efficiency (NUE) was achieved by treatment T_5 ($H_2S_1N_1$: NS1250 hybrid at 45 cm × 45 cm spacing with 120:80:60 kg NPK ha^{-1}), registering a value of 56.38 kg kg^{-1} (Table 5). This treatment also led in Phosphorus Use Efficiency (PUE), recording 38.64 kg kg^{-1} and Potassium Use Efficiency (KUE) with 90.51 kg kg^{-1}. In contrast, treatment T_3 ($H_1S_2N_1$: Lucky hybrid at 60 cm × 45 cm spacing with 120:80:60 kg NPK ha^{-1}) exhibited the lower nutrient efficiency across all three macronutrients with NUE at 25.85 kg kg^{-1}, PUE at 23.23 kg kg^{-1} and KUE at 45.45 kg kg^{-1}. The higher nutrient use efficiency in treatment T_5 is attributed to the NS1250 hybrid's strong genetic potential for effective nutrient utilization, combined with closer spacing (45 cm × 45 cm), which promotes a denser plant population and maximizes nutrient uptake per unit of fertilizer applied. Therefore, the synergy of a nutrient-efficient hybrid, optimal plant density and appropriate nutrient supply in T_5 results in superior nutrient use efficiency. Comparable results were observed by Kumar *et al.* (2018), Yildirim *et al.* (2020), Hussain *et al.* (2021) and Abhijithnaik *et al*. (2023) in broccoli.

Available Micronutrients

The interaction effects of hybrids, plant spacing, major nutrient levels on the available iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and boron (B) in the soil were found non-significant. The corresponding data are presented in Table 6.

Micronutrients Uptake

The uptake of essential micronutrients- zinc (Zn), iron (Fe), manganese (Mn), copper (Cu) and boron (B) by broccoli hybrids was evaluated under open field condition. The interaction effect of different hybrids, plant spacing and nutrient levels on micronutrient

TABLE 6

Interaction effect of hybrids, plant spacing and nutrient levels on available micronutrients in soil at harvest

Treatment	Zn (mg ha^{-1})	Fe (mg ha^{-1})	Mn (mg ha^{-1})	Cu (mg ha^{-1})	B (mg ha^{-1})
$T_1 – H_1S_1N_1$	0.34	4.15	3.48	3.01	0.27
$T_2 – H_1S_1N_2$	0.34	4.15	3.46	3.07	0.26
$T_3 – H_1S_2N_1$	0.34	4.16	3.44	3.01	0.27
$T_4 – H_1S_2N_2$	0.34	4.16	3.45	3.05	0.28
$T_5 – H_2S_1N_1$	0.35	4.15	3.48	3.04	0.26
$T_6 – H_2S_1N_2$	0.33	4.13	3.47	3.06	0.26
$T_7 – H_2S_2N_1$	0.33	4.15	3.47	3.01	0.27
$T_8 – H_2S_2N_2$	0.34	4.16	3.48	3.05	0.26
$T_9 – H_3S_1N_1$	0.33	4.12	3.41	3.05	0.26
$T_{10} – H_3S_1N_2$	0.33	4.15	3.44	3.03	0.28
$T_{11} – H_3S_2N_1$	0.34	4.15	3.42	3.02	0.26
$T_{12} – H_3S_2N_2$	0.33	4.16	3.45	3.04	0.27
F-test	NS	NS	NS	NS	NS
S.Em±	0.01	0.02	0.05	0.03	0.01
CD (5%)	-	-	-	-	-

*Significance @ 5% level, NS – Non significant

H_1: Lucky; H_2: NS1250; H_3: Saki; S_1: 45 cm × 45 cm, S_2: 60 cm × 45 cm; N_1: 120:80:60 kg NPK ha$^{-1;}$ N_2: 150:100:75 kg NPK ha^{-1}

TABLE 7

Interaction effect of hybrids, plant spacing and nutrient levels on micronutrient uptake by broccoli hybrids

Treatment	Zn (mg ha^{-1})	Fe (mg ha^{-1})	Mn (mg ha^{-1})	Cu (mg ha^{-1})	B (mg ha^{-1})
$T_1 – H_1S_1N_1$	78.02	191.08	106.61	39.14	54.34
$T_2 – H_1S_1N_2$	78.17	195.38	105.71	38.17	54.69
$T_3 – H_1S_2N_1$	79.22	196.75	106.31	38.54	54.74
$T_4 – H_1S_2N_2$	79.57	194.72	107.76	39.79	55.10
$T_5 – H_2S_1N_1$	80.21	204.58	108.30	40.94	55.31
$T_6 – H_2S_1N_2$	83.98	211.42	111.97	40.81	56.45
$T_7 – H_2S_2N_1$	81.76	215.43	111.50	42.11	55.97
$T_8 – H_2S_2N_2$	85.17	214.28	112.12	41.89	57.35
$T_9 – H_3S_1N_1$	81.22	199.68	110.39	40.12	55.36
$T_{10} – H_3S_1N_2$	85.02	206.53	111.99	41.27	57.09
$T_{11} – H_3S_2N_1$	83.17	196.58	109.47	40.01	56.11
$T_{12} – H_3S_2N_2$	86.14	208.47	110.23	42.57	61.02
F-test	NS	NS	NS	NS	NS
S.Em±	1.08	2.47	1.55	0.67	0.94
CD (5%)	-	-	-	-	-

*Significance @ 5% level, NS – Non significant

H_1: Lucky; H_2: NS1250; H_3: Saki; S_1: 45 cm × 45 cm, S_2: 60 cm × 45 cm; N_1: 120:80:60 kg NPK ha$^{-1;}$ N_2: 150:100:75 kg NPK ha^{-1}

uptake is presented in Table 7. The measured values of the uptake of micronutrients, were not statistically significant.

The study revealed that the broccoli hybrid NS1250 (H_2) consistently exhibited the higher nutrient uptake and lower residual soil nutrient levels after harvest. Wider plant spacing (60 cm × 45 cm) resulted in greater residual soil nutrients and nutrient uptake compared to closer spacing (45 cm × 45 cm). Higher nutrient application rates (150:100:75 kg NPK ha^{-1}) enhanced both nutrient availability in the soil after harvest and uptake by plants. Significant interactions among hybrids, plant spacing and nutrient levels influenced nutrient dynamics with the combination of Lucky hybrid, wider spacing and higher nutrient dose ($H_1S_2N_2$) producing the higher residual soil nutrients, and NS1250 with wider spacing and both nutrient rates ($H_2S_2N_1$ and $H_2S_2N_2$) achieving the greater nutrient uptake. Overall, treatment T_8 proved most effective for maximizing nutrient uptake, as wider spacing combined with higher nutrient supply allowed the plants to access more resources, resulting in significantly greater absorption of nitrogen, phosphorus and potassium compared to other treatments.

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Evaluation of Fertilizer Recommendation Approaches on Maize Growth, Yield and Yield Attributes in *Alfisols* of Karnataka

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Abstract

Site-specific targeted fertilizer recommendations integrated with organic amendments and nano-fertilizers offer a promising strategy to enhance nutrient use efficiency and maize productivity. In *Rabi* 2024 verification trials at ZARS, GKVK (low, medium and high fertility strips) and farmers' fields in the Southern Transition Zone (Shivamogga), seven nutrient management approaches were compared each other. The treatments are, STCR NPK (targeted yield 100 and 90 q ha^{-1}) with and without farmyard manure and nano-DAP, general recommended dose, soil fertility rating and absolute control. Growth (plant height, leaf number, leaf area index), physiological (chlorophyll meter readings) and yield attributes (cob length, kernels per row, rows per cob, test weight) were recorded at 30, 60, 90 DAS and at harvest. Kernel and stover yields were estimated at harvest. Data were analysed using ANOVA and DMRT. STCR NPK + FYM targeted for 100 q ha^{-1} (T_2) consistently produced superior vegetative growth, higher chlorophyll readings and larger LAI across fertility strips and the farmers' field. Treatment T_2 (T_2 : STCR NPK + FYM Targeted Yield 100 q ha^{-1}) recorded the highest kernel and stover yields, significantly outperforming GRD, SFR and control. Other STCR variants (T_1, T_3, T_4) were comparable but generally numerically lower. Yield components including cob length, kernels per row and rows per cob were noted maximum under T_2, while test weight was also higher under STCR + FYM at most sites. The enhanced performance under STCR + FYM is attributed to balanced, target-oriented nutrient supply, improved soil physical especially soil moisture and biological properties through FYM and improved nutrient synchrony aided by nano-DAP. These findings demonstrated that STCR integrated with organic inputs and nano-fertilizers can increase maize productivity in *Alfisols* of Karnataka.

Keywords : Maize, Farmyard manure, STCR, Nano-DAP, Maize productivity, Site-specific nutrient management

Maize (*Zea mays* L.) is a major cereal crop cultivated across tropical and subtropical regions, valued for its versatility as food, feed and industrial raw material (Dey *et al*., 2024). Its adaptability to diverse agro-climatic conditions, short duration and high yield potential make it a vital component of India's agricultural economy (Murdia *et al.*, 2016). Beyond human consumption, maize supports livestock and poultry industries, while also serving as a raw material for starch, ethanol and other agro-industrial products (Shiferaw *et al*., 2011). However, achieving optimal yields in maize requires effective nutrient management, which is influenced by soil fertility levels, crop requirements and external nutrient inputs.

Nutrient management in maize has traditionally relied on generalized fertilizer recommendations or

farmer practices, which prescribe uniform doses of fertilizers irrespective of site-specific soil conditions (Khan, 2024). These blanket recommendations often overlook inherent variability in soil fertility, leading to over or under-application of nutrients. Such imbalances can reduce crop yield, increase input costs and deteriorate soil health over time. To improve in nutrient management approaches such as Low, Medium and High (LMH) nutrient applications were developed, where fertilizer doses are adjusted according to soil fertility categories. While more refined than blanket doses, the LMH approach still lacks the precision required for site-specific nutrient management (Krishna Murthy *et al*., 2024c).

In response to these limitations, the Soil Test Crop Response (STCR) approach has emerged as a scientifically robust method of fertilizer recommendation. STCR prescriptions are based on actual soil test values, crop nutrient requirements and defined yield targets, allowing for more accurate and efficient nutrient application (Basavaraj *et al*., 2014). This approach not only enhances maize productivity but also improves nutrient use efficiency, thereby reducing losses through leaching, volatilization and runoff, which are common under conventional practices (Govindasamy *et al*., 2023).

The major advantage of STCR lies in its ability to generate targeted yield equations that relate soil nutrient availability and crop response to applied fertilizers, thereby optimizing nutrient inputs for achieving predetermined yield goals. This contrasts sharply with LMH or generalized recommendations, which disregard actual soil nutrient reserves and crop-specific nutrient uptake dynamics (Krishnamurthy *et al*., 2023). As a result, STCR-based fertilizer recommendations often outperform traditional approaches by synchronizing nutrient supply with crop demand of maize during critical growth stages, leading to improved yields and resource-use efficiency (Ashok *et al*., 2013).

Post-harvest soil nutrient availability in maize systems is strongly influenced by initial soil fertility, fertilizer application and crop nutrient uptake. Continuous cultivation under blanket or LMH recommendations can deplete soil reserves, while STCR-based recommendations balance nutrient removal and replenishment, thereby maintaining soil fertility (Suresh and Santhi, 2018). Recent advances also include the integration of organic manures and nano-fertilizers into STCR models, further improving nutrient recovery and soil health (Marella *et al.,* 2021 and Pudhuvai *et al.,* 2024).

Therefore, evaluating the performance of different fertilizer recommendation approaches - including general recommendation, LMH and STCR - in maize systems is essential. Such comparisons not only validate the accuracy of STCR equations but also highlight their superiority in sustaining productivity, nutrient use efficiency and profitability under *Alfisol* conditions. With this brief background, a study on 'Evaluation of Fertilizer Recommendation Approaches on Maize Growth, Yield and Yield Attributes in *Alfisols* of Karnataka' was conducted with a major objective to compare the effectiveness of different nutrient recommendation approaches such as general recommended dose (GRD), low-medium-high and soil test crop response in maize cultivation under *Alfisol* conditions.

Material and Methods

Verification Trial

The verification trials with maize were conducted during *Rabi* 2024 to validate the fertilizer prescription equations developed through the Soil Test Crop Response approach for growth and yield parameters of maize. These trials were laid out both at the Zonal Agricultural Research Station (ZARS), GKVK, Bengaluru and at farmers fields in the Southern Transition Zone, Shivamogga. The experiments were designed to test the efficacy of different nutrient recommendation approaches under varying fertility situations.

Location and Site Details

At ZARS, GKVK, the verification trials were conducted in Low Fertility Strip (LFS), Medium Fertility Strip (MFS) and High Fertility Strip (HFS) developed through fertility gradient experiments. The station is located at 13° 04' 55.23" N latitude and

77° 34' 10.03" E longitude, at an altitude of 930 m above mean sea level (MSL), falling in the Eastern Dry Zone (Zone 5) of Karnataka. The parallel verification trial at farmers fields in Shivamogga was conducted in the Southern Transition Zone (Zone 7), situated at 14° 03' 06.93" N latitude and 75° 14' 54.73" E longitude, with an altitude of 654 m above MSL.

Crop and Season

The test crop was maize hybrid *var.* MAH-14-5, sown during *Rabi* 2024. The crop was planted at a spacing of 60 cm × 30 cm in plots of 12.10 m² at ZARS and 13.5 m² at the farmer's field. The date of sowing was 20th September 2024 at ZARS and 5th October 2024 at Shivamogga. The crop was harvested on 17th January 2025 and 3rd February 2025, respectively.

Experimental Conditions

At ZARS, GKVK, the total rainfall received during the crop growth period was 724.6 mm. Minimum air temperatures ranged between 15.2-19.6 °C and maximum temperatures between 26.9-29.4 °C. Relative humidity varied from 53-67% (minimum) and 86-89 per cent (maximum). Bright sunshine hours ranged from 4.3 to 8.1 hr and potential evapotranspiration (PET) varied from 3.3-4.5 mm (Fig. 1).

At Shivamogga (Farmers Field), The total rainfall received was 178.4 mm. The minimum temperature ranged from 15.2-20.0 °C and maximum from 27.4-32.2 °C. Relative humidity varied between 45-66% (minimum) and 80-88% (maximum). Bright sunshine hours ranged between 5.7-9.2 hr, while PET varied between 3.7-4.8 mm (Fig. 2).

The verification trials were undertaken to validate the applicability of STCR-based targeted yield equations for maize under real field conditions. These trials specifically aimed to compare the performance of different nutrient recommendation approaches, including recommended dose of fertilizers (GRD), low-medium-high (LMH) and STCR-based prescriptions with or without the integration of farmyard manure. By evaluating these approaches simultaneously at the research station and in farmers fields, the study sought to determine their relative effectiveness in enhancing maize growth, yield and nutrient use efficiency. The key focus was on assessing whether the targeted yield prescriptions

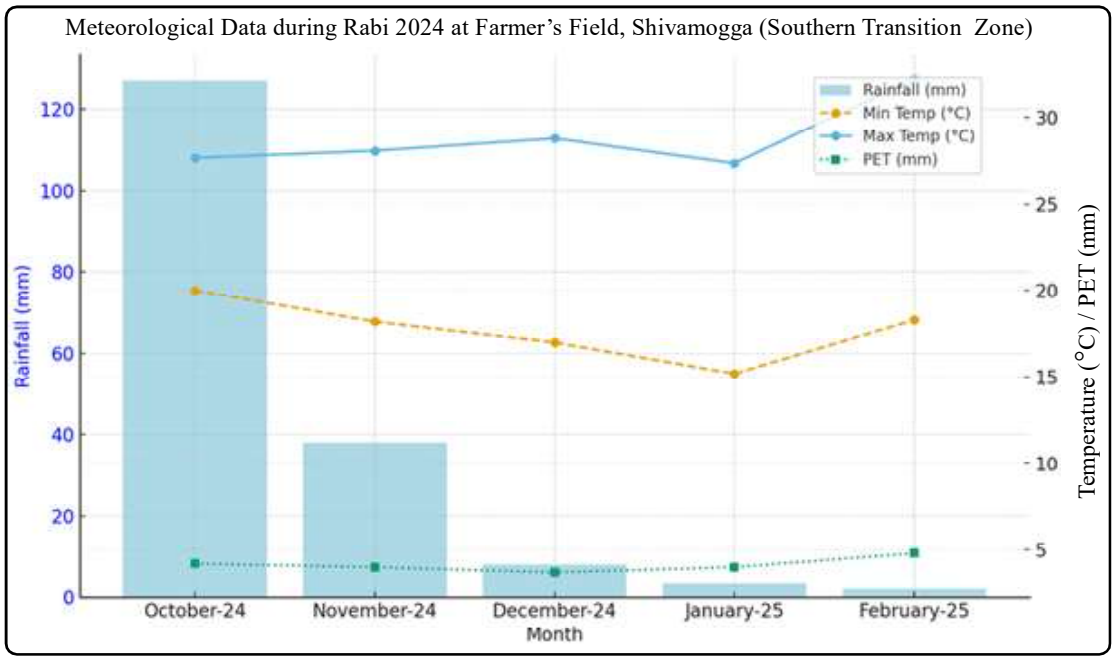

Fig. 1 : Meteorological data of the verification trial experimental site during *Rabi* 2024 at ZARS, GKVK, Bengaluru

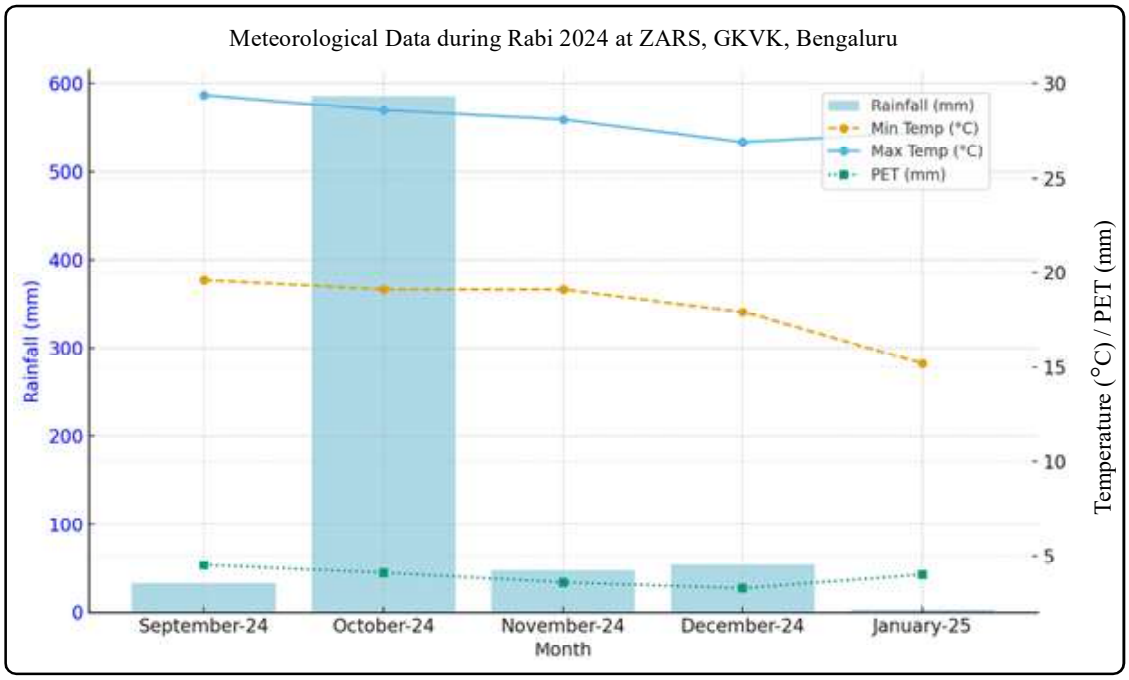

Fig. 2 : Meteorological data of the verification trial experimental site during Rabi 2024 at farmers field, Southern Transition Zone, Shivamogga

could achieve the expected productivity levels within a permissible deviation of ±10 per cent, thereby ensuring their reliability and practical utility for farmers across varying soil fertility situations.

Soil Characteristics of the Experimental Site

A composite soil sample was collected from 0-20 cm depth of each plot after laying out the plan but before the commencement of experiment. The collected soil samples were air dried, pounded and passed through a 2 mm sieve and was analyzed for physical and chemical properties. The results of physical and chemical properties of soils are presented in Table 1.

Design and Layout of the Experiment

The experiment was laid out in randomized complete block design having seven treatments and replicated thrice. The plan of layout of the verification trial is shown in Fig. 3.

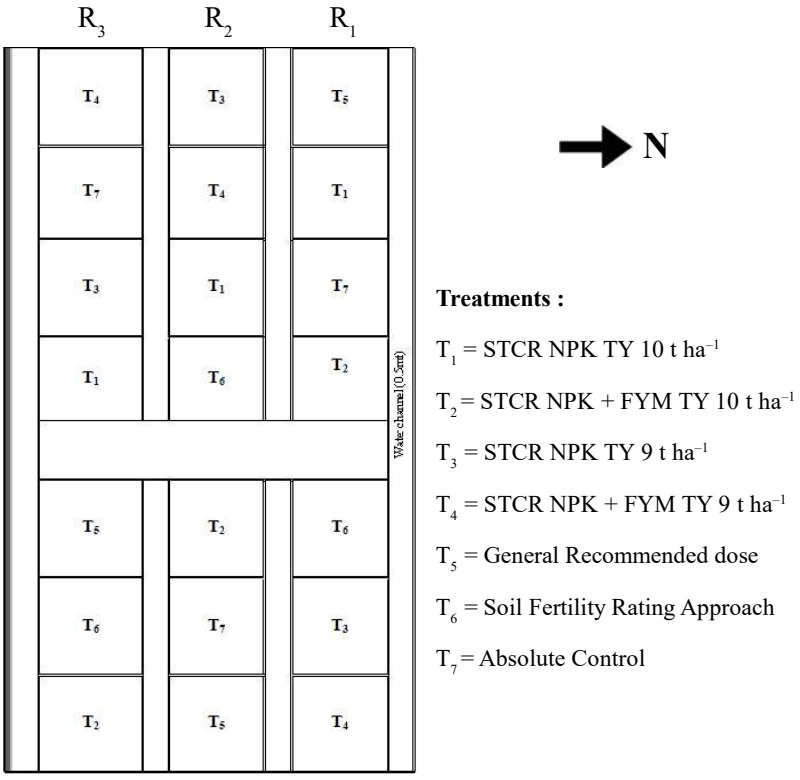

Fig. 3 : Layout plan of the verification trial

Note : Same layout of low fertility strip followed for medium, high fertility strip and farmers field of southern transition zone of shivamogga

Table 1

Initial soil physico-chemical characteristics of the verification trials of ZARS, Gandhi Krishi Vignyan Kendra, Bengaluru and Farmers fields in the Southern Transition Zone, Shivamogga

Particulars	ZARS, GKVK, Bengaluru			Farmers fields in the Southern Transition Zone, Shivamogga
	LFS	MFS	HFS	
Physical properties of soil				
Sand (%)	68.50	67.95	67.90	68.40
Silt (%)	13.40	13.55	13.30	12.40
Clay (%)	18.10	18.50	18.80	19.20
Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Bulk Density (Mg m^{-3})	1.35	1.33	1.31	1.35
Maximum water holding capacity (%)	35.83	6.47	37.13	9.40
Chemical properties of soil				
pH (1:2.5)	6.29	6.25	6.21	6.01
Electrical conductivity (dS m^{-1})	0.22	0.25	0.31	0.08
Organic carbon (g kg^{-1})	4.70	4.72	4.78	10.90
Available N (kg ha^{-1})	195.57	237.87	305.97	489.55
Available P (kg ha^{-1})	215.01	372.66	447.03	27.83
Available K (kg ha^{-1})	147.64	239.04	315.86	201.84

Fertilizer Schedule

Nitrogen, phosphorus and potassium were applied through urea, SSP and MOP. 75% of RDN was supplied through conventional fertilizers (50% of 75% RDN at sowing, 25% of 75% RDN at 45 DAS and remaining 25% of 75% RDN at 75 DAS) and foliar application of Nano fertilizers at 30 DAS and 60 DAS through Nano DAP @ 4mL L^{-1}. 75% of RDP was supplied through conventional fertilizers at basal and foliar application of Nano DAP at 30 DAS and 60 DAS @4 mL L^{-1}. 100% of RDK was supplied through conventional fertilizers at basal.

Treatment details :

T_1 : STCR NPK Targeted Yield 100 q ha^{-1}

T_2 : STCR NPK + FYM Targeted Yield 100 q ha^{-1}

T_3 : STCR NPK Targeted Yield 90 q ha^{-1}

T_4 : STCR NPK + FYM Targeted Yield 90 q ha^{-1}

T_5 : General Recommended dose

T_6 : Soil Fertility Rating Approach

T_7 : Absolute Control

Biometric Observations

Biometric observations on various growth parameters at 30 DAS, 60 DAS, 90 DAS and at harvest and various yield parameters after harvest of crop were recorded on five randomly selected and tagged representative plant hills in each plot and expressed as mean values.

Growth Parameters

Plant growth parameters were recorded at different growth stages of maize. Plant height was measured from ground level to the base of the flag leaf at vegetative stages and to the tip of the tassel at maturity from five randomly selected plants and the mean was expressed in centimeters (Ghaffari *et al*., 2011). The

total number of fully expanded leaves per plant was counted at the peak vegetative stage from five randomly selected plants and the average was recorded (Ghaffari *et al*., 2011). Leaf area was determined using a Leaf Area Meter by selecting three representative plants per plot, measuring all fully expanded leaves and expressing the mean value in square centimeters (cm^2) (Ghaffari *et al*., 2011). Chlorophyll content was estimated using a Field Scout Chlorophyll Meter (CM 1000), where three readings were taken from each plant at different leaf positions and averaged. A CM 1000 reading of 250 corresponds to a SPAD value of 41.60, equivalent to 1.90 mg g^{-1} of chlorophyll content (Mahajan *et al*., 2014).

Yield Parameters

Yield and yield-attributing characters of maize were recorded following standard procedures. Cob length was measured from base to tip (excluding the peduncle) from three randomly selected cobs and expressed in centimetres, while the number of kernel rows per cob and the number of kernels per row were counted manually from the same cobs and the respective averages were calculated (Ghaffari *et al*., 2011). Test weight was determined by counting one hundred kernels from the harvested lot of each plot, weighing them on a digital balance and expressing the value in grams (Ghaffari *et al*., 2011). Kernel yield was obtained by manually shelling the harvested cobs from each plot, sun drying, cleaning and weighing the kernels and then converting the net plot yield into quintals per hectare (q ha^{-1}). Stover yield was recorded after removing the cobs, sun drying the stover for about 10 days and computing the yield per hectare from plot-wise data, expressed in q ha^{-1} (Ghaffari *et al*., 2011).

Statistical analysis of data

Experimental data generated in the verification trial was subjected to statistical analysis adopting Fisher's method of analysis of variance as outlined by Krishna Murthy *et al.* (2024a). The level of significance used in 'F' and 't' test was 5 per cent. Critical difference (CD) values were calculated at 5 per cent level of significance whenever 'F' test was found significant. Post-harvest soil test value prediction equations were developed by multiple regression analysis from SPSS 16.0 software.

Results and Discussion

Influence of Various Approaches of Fertilizer Recommendation on Growth Parameters of Maize

Plant Height

The data on plant height (cm) of maize recorded at 30, 60 and 90 DAS, as well as at harvest, under different nutrient management approaches are presented in Table 2.

Maize plant height was markedly affected by the various fertilizer prescription approaches across all growth stages, namely 30, 60 and 90 DAS, as well as at harvest. Among the treatments, significantly higher plant height was noticed in STCR NPK + FYM (100 q ha^{-1}) (T_2: 78.12 cm, 167.17 cm, 192.25 cm and 198.17 cm in LFS, 81.22 cm, 192.67 cm, 219.64 cm, 225.66 cm in MFS and 86.33 cm, 189.07 cm, 215.54 cm and 220.33 cm in HFS, respectively) compared to soil fertility rating approach (T_6: 65.00 cm, 146.33 cm, 168.27 cm and 174.10 cm, respectively in LFS, 71.00 cm, 150.33 cm, 171.38 cm and 177.40 cm, respectively in MFS and 65.02 cm, 158.13 cm, 180.27 cm and 185.06 cm, respectively in HFS), general recommended dose (T_5: 63.08 cm, 142.00 cm, 163.63 cm and 169.19 cm, respectively in LFS, 70.78, 145.67, 166.06 and 172.08 cm, respectively in MFS and 62.67 cm, 155.23 cm, 176.96 cm and 181.75 cm, respectively in HFS) and absolute control (T_7: 60.67 cm, 117.33 cm, 134.83 cm and 145.83 cm, respectively in LFS, 66.22 cm, 132.67 cm, 151.50 cm and 158.17 cm, respectively in MFS and 60.90 cm, 143.47 cm, 164.56 cm and 168.00 cm, respectively in HFS). However, STCR treatments were on par with each other.

The study revealed significant difference in maize plant height across all growth stages under various nutrient management approaches in farmers fields of the southern transition zone, Shivamoga. The STCR NPK + FYM treatment for the 100 q ha^{-1} yield

TABLE 2

Influence of different approaches of nutrient application on plant height of maize at different intervals of crop growth

Treatment details		Plant height (cm)															
		LFS				MFS				HFS				Farmer field, STZ, Shivamogga			
		30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest
T_1	STCR NPK Targeted Yield 100 q ha^{-1}	76.01	157.83	181.50	187.39	77.89	184.00	210.09	215.78	83.67	184.17	209.95	214.74	78.40	184.53	210.60	216.30
T_2	STCR NPK + FYM Targeted Yield 100 q ha^{-1}	78.12	167.17	192.25	198.17	81.22	192.67	219.64	225.66	86.33	189.07	215.54	220.33	81.73	193.53	220.17	226.17
T_3	STCR NPK Targeted Yield 90 q ha^{-1}	72.25	154.00	177.13	182.99	77.89	168.33	191.90	197.92	72.33	170.27	194.11	198.90	75.40	168.83	192.43	198.43
T_4	STCR NPK + FYM Targeted Yield 90 q ha^{-1}	74.31	155.50	178.83	184.72	78.00	176.33	201.02	208.04	75.67	176.07	200.72	205.51	78.53	179.87	203.53	207.57
T_5	General Recommended dose	63.08	142.00	163.63	169.19	70.78	145.67	166.06	172.08	62.67	155.23	176.96	181.75	68.30	146.20	166.57	172.57
T_6	Soil Fertility Rating Approach	65.00	146.33	168.27	174.10	71.00	150.33	171.38	177.40	65.02	158.13	180.27	185.06	69.07	150.87	171.90	177.93
T_7	Absolute control	60.67	117.33	134.83	145.83	66.22	132.67	151.50	158.17	60.90	143.47	164.56	168.00	62.93	135.10	152.77	158.00
	S.Em. ±	3.16	7.08	8.14	8.44	3.53	6.36	6.74	9.41	4.55	5.30	7.16	9.45	3.48	8.05	9.17	9.43
	C.D. @ 5 %	9.00	20.17	23..19	24.04	10.05	18.13	19.20	26.82	12.96	15.09	20.40	26.92	9.93	22.94	26.12	26.88

TABLE 3

Influence of different approaches of nutrient application on number of leaves of maize at different intervals of crop growth

Treatment details		Number of leaves per plant															
		LFS				MFS				HFS				Farmer field, STZ, Shivamogga			
		30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest
T_1	STCR NPK Targeted Yield 100 q ha^{-1}	6.44	11.67	10.89	10.23	6.67	12.22	11.25	10.95	7.11	12.56	11.56	10.90	6.70	13.40	12.00	11.80
T_2	STCR NPK + FYM Targeted Yield 100 q ha^{-1}	6.89	12.67	11.67	11.00	6.56	13.00	12.68	12.35	8.00	13.56	12.56	11.90	6.87	13.80	12.50	12.37
T_3	STCR NPK Targeted Yield 90 q ha^{-1}	6.11	11.67	11.23	10.57	5.89	12.56	11.00	10.67	7.11	12.78	11.78	11.10	6.40	12.70	11.10	10.85
T_4	STCR NPK + FYM Targeted Yield 90 q ha^{-1}	6.56	12.22	10.67	10.00	6.78	12.00	11.89	11.57	7.04	13.11	12.11	11.43	6.55	13.00	12.01	11.30
T_5	General Recommended dose	6.56	12.89	11.00	10.33	6.44	12.33	11.18	10.90	7.11	11.89	12.00	11.33	5.90	11.70	10.00	10.00
T_6	Soil Fertility Rating Approach	6.89	12.00	10.89	10.23	7.22	12.22	11.19	11.10	7.67	12.06	11.89	11.23	6.00	11.90	10.30	10.30
T_7	Absolute control	6.00	9.78	9.23	8.70	6.00	10.56	9.11	8.77	7.05	12.11	11.11	10.45	6.01	10.77	8.70	8.70
	S.Em. ±	0.31	0.54	0.50	0.47	0.37	0.57	0.52	0.50	0.39	0.51	0.55	0.52	0.30	0.60	0.53	0.52
	C.D. @ 5 %	NS	1.55	1.44	1.35	NS	1.61	1.48	1.44	NS	NS	NS	NS	0.85	1.70	1.50	1.48

target (T_2) consistently produced the highest plant height, recording 81.73 cm at 30 DAS, 193.53 cm at 60 DAS, 220.17 cm at 90 DAS and 226.17 cm at harvest. These values were statistically superior to other approaches, including the soil fertility rating method (T_6: 69.07, 150.87, 171.90 and 177.93 cm), general recommended dose (T_5: 68.30, 146.20, 166.57 and 172.57 cm), and absolute control (T_7: 62.93, 135.10, 152.77 and 158.00 cm) at the respective growth stages. Other STCR-based nutrient recommendations showed on par results with T_2 [STCR NPK + FYM (100 q ha^{-1})] except T_3 [STCR NPK (90 q ha^{-1})] at 60, 90 DAS and at harvest recorded 168.83, 192.43 and 198.43 cm, respectively.

Number of Leaves Per Plant

The number of leaves per plant varied with different nutrient management practices at all growth stages (30, 60, 90 DAS and at harvest) across fertility strips and locations (Table 3). At 30 DAS, the differences among treatments were statistically non-significant in all fertility srtips; however, significant differences were observed at later stages except in farmer field of Shivamogga.

Among the treatments, T_2 [STCR NPK + FYM (100 q ha^{-1})] recorded significantly higher number of leaves per plant across all growth stages. In LFS, T_2 [STCR NPK + FYM (100 q ha^{-1})] recorded 6.89, 12.67, 11.67 and 11.00 leaves at 30, 60, 90 DAS and harvest, respectively. In MFS, it recorded 6.56, 13.00, 12.68 and 12.35 leaves at 30, 60, 90 DAS and harvest, respectively and in HFS, it showed 8.00, 13.56, 12.56 and 11.90 leaves at 30, 60, 90 DAS and harvest, respectively. These values were significantly higher compared to T_7 (absolute control) treatment, which recorded comparatively fewer leaves at all stages.

The farmer field of Shivamogga recorded significantly higher number of leaves per plant under T_2 [STCR NPK + FYM (100 q ha^{-1})] with 6.87, 13.80, 12.50 and 12.37 leaves at 30, 60, 90 DAS and harvest, respectively, which was significantly higher compared to T_6 [soil fertility rating approach] (6.00, 11.90, 10.30 and 10.30 leaves at 30, 60, 90 DAS and harvest, respectively), T_5 [general recommended dose] (5.90, 11.70, 10.00 and 10.00 leaves at 30, 60, 90 DAS and harvest, respectively) and T_7 [absolute control] (6.01, 10.77, 8.70 and 8.70 leaves at 30, 60, 90 DAS and harvest, respectively). Other STCR-based nutrient recommendation approaches showed comparable results with T_2 [STCR NPK + FYM (100 q ha^{-1})] but were slightly lower, particularly at 90 DAS and harvest.

Chlorophyll Meter Reading

Chlorophyll meter readings of maize leaves were significantly influenced by different fertilizer management approaches at various crop growth stages (30, 60, 90 DAS and at harvest) across all locations and fertility strips (Table 4).

Across the treatments, T_2 [STCR NPK + FYM (100 q ha^{-1})] recorded higher chlorophyll meter readings throughout the crop growth period. In LFS, T_2 [STCR NPK + FYM (100 q ha^{-1})] (252.33, 345.44, 291.67 and 250.78 at 30, 60, 90 DAS and harvest, respectively). These were significantly higher than T_7 [absolute control] (189.89 and 230.22 at 30 and 60 DAS, respectively). The same treatment, T_2 [STCR NPK + FYM (100 q ha^{-1})] recorded significantly higher values than T_6 [soil fertility rating approach], T_5 [general recommended dose] and T_7 [absolute control] at 90 DAS and at harvest in LFS. In MFS, the T_2 [STCR NPK + FYM (100 q ha^{-1})] values were 284.56, 362.78, 315.51 and 273.56 at 30, 60, 90 DAS and harvest, respectively and in HFS, the readings were 290.22, 398.44, 298.13 and 255.22 at 30, 60, 90 DAS and harvest, respectively, which were significantly higher compared to T_7 [absolute control], which showed lower chlorophyll meter readings of 189.89, 230.22, 212.89 and 172.00 at 30, 60, 90 DAS and harvest, respectively in LFS; 189.33, 263.67, 208.18 and 166.22 at 30, 60, 90 DAS and harvest, respectively in MFS and 218.22, 246.00, 251.58 and 208.67 in HFS at 30, 60, 90 DAS and harvest, respectively.

Across locations, the farmer field of Shivamogga recorded significantly higher chlorophyll meter values under T_2 [STCR NPK + FYM (100 q ha^{-1})] with

TABLE 4
Influence of different approaches of nutrient application on Chlorophyll meter reading of maize at different intervals of crop growth

Treatment details		Chlorophyll meter reading															
		LFS				MFS				HFS				Farmer field, STZ, Shivamogga			
		30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest
T_1	STCR NPK Targeted Yield 100 q ha^{-1}	237.00	341.00	263.89	241.00	271.44	345.33	287.85	245.89	267.56	354.11	275.58	232.67	270.33	360.67	325.00	260.33
T_2	STCR NPK + FYM Targeted Yield 100 q ha^{-1}	252.33	345.44	291.67	250.78	284.56	362.78	315.51	273.56	290.22	398.44	298.13	255.22	285.46	372.33	340.96	278.17
T_3	STCR NPK Targeted Yield 90 q ha^{-1}	246.44	341.44	259.82	231.44	278.78	359.22	278.29	242.33	271.56	380.22	267.05	239.56	260.33	345.00	305.33	246.00
T_4	STCR NPK + FYM Targeted Yield 90 q ha^{-1}	247.22	350.89	260.78	236.00	281.89	360.11	280.96	243.00	280.56	382.44	270.80	251.22	265.33	356.67	315.00	251.67
T_5	General Recommended dose	244.33	340.11	251.11	210.22	257.89	319.67	300.73	268.78	267.33	391.78	262.67	249.00	240.33	310.00	275.33	215.00
T_6	Soil Fertility Rating Approach	248.44	344.89	252.56	221.67	261.78	334.67	292.53	264.56	274.33	389.22	266.86	251.78	245.67	320.00	286.67	225.00
T_7	Absolute control	189.89	230.22	212.89	172.00	189.33	263.67	208.18	166.22	218.22	246.00	251.58	208.67	192.33	264.33	236.00	173.49
	S.Em. ±	11.26	15.07	11.68	9.93	12.23	15.71	13.07	11.23	12.43	16.70	12.74	11.07	12.02	15.92	14.34	11.41
	C.D. @ 5 %	32.08	42.94	33.29	28.29	34.85	44.78	37.24	32.01	35.41	47.60	36.30	31.55	34.25	45.38	40.87	32.52

TABLE 5

Influence of different approaches of nutrient application on leaf area index of maize at different intervals of crop growth

Treatment details	Leaf area index															
	LFS				MFS				HFS				Farmer field, STZ, Shivamogga			
	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest
T_1 STCR NPK Targeted Yield 100 q ha^{-1}	0.33	2.74	3.34	1.57	0.33	2.77	3.40	1.67	0.34	2.79	3.46	1.73	0.35	2.74	3.41	1.69
T_2 STCR NPK + FYM Targeted Yield 100 q ha^{-1}	0.36	2.98	3.63	1.71	0.36	3.01	3.70	1.82	0.37	3.03	3.76	1.88	0.38	2.98	3.71	1.84
T_3 STCR NPK Targeted Yield 90 q ha^{-1}	0.30	2.31	2.92	1.33	0.28	2.34	2.88	1.41	0.29	2.37	2.94	1.47	0.30	2.31	2.89	1.43
T_4 STCR NPK + FYM Targeted Yield 90 q ha^{-1}	0.34	2.69	3.28	1.54	0.34	2.82	3.47	1.70	0.34	2.84	3.52	1.76	0.36	2.79	3.48	1.72
T_5 General Recommended dose	0.27	2.14	2.61	1.23	0.27	2.24	2.75	1.35	0.27	2.27	2.82	1.41	0.29	2.21	2.76	1.37
T_6 Soil Fertility Rating Approach	0.28	2.21	2.69	1.27	0.28	2.32	2.85	1.40	0.28	2.34	2.90	1.45	0.30	2.29	2.86	1.42
T_7 Absolute control	0.27	0.86	0.93	0.97	0.30	0.87	1.10	0.57	0.31	1.01	1.23	0.65	0.31	0.84	1.12	0.60
S.Em. ±	0.01	0.12	0.14	0.07	0.01	0.12	0.14	0.07	0.02	0.12	0.15	0.07	0.01	0.11	0.13	0.07
C.D. @ 5 %	0.04	0.33	0.40	0.19	0.04	0.34	0.41	0.20	0.04	0.34	0.42	0.21	0.04	0.30	0.38	0.19

285.46, 372.33, 340.96 and 278.17 at 30, 60, 90 DAS and harvest, respectively. These were significantly higher than the values recorded under T_6 [soil fertility rating approach] (245.67, 320.00, 286.67 and 225.00), T_5 [general recommended dose] (240.33, 310.00, 275.33 and 215.00) and T_7 [absolute control] (192.33, 264.33, 236.00 and 173.49). Other STCR-based nutrient recommendation treatments showed readings on par with T_2 [STCR NPK + FYM (100 q ha^{-1})] in the early phase of crop growth but were comparatively lower at 90 DAS and at harvest.

Leaf Area Index (LAI)

The LAI of maize was significantly influenced by different fertilizer management approaches across the growth stages in different fertility strips (Table 5).

Among the treatments in LFS, T_2 [STCR NPK + FYM (100 q ha^{-1})] recorded significantly higher LAI across growth stages, with values of 0.36 at 30 DAS, 2.98 at 60 DAS, 3.63 at 90 DAS and 1.71 at harvest, compared to T_5 [general recommended dose] (0.27, 2.14 and 2.63 at 30, 60 DAS and at harvest), T_6 [soil fertility rating approach] (0.28, 2.21 and 1.27 at 30, 60 DAS and at harvest) and T_7 [absolute control] (0.27, 0.86, 0.93 and 0.97, respectively at 30, 60, 90 DAS and at harvest). T_4 [STCR NPK + FYM (90 q ha^{-1})] and T_1 [STCR NPK (100 q ha^{-1})] were statistically on par with T_2 [STCR NPK + FYM (100 q ha^{-1})] at 60 and 90 DAS but recorded significantly lower LAI than T_2 [STCR NPK + FYM (100 q ha^{-1})] at harvest. Other treatments like T_3 [STCR NPK (90 q ha^{-1})], T_5 [general recommended dose] and T_6 [soil fertility rating approach] recorded significantly lower LAI than T_2 [STCR NPK + FYM (100 q ha^{-1})] at 30, 60 DAS and at harvest.

In MFS, among the treatments, T_2 [STCR NPK + FYM (100 q ha^{-1})] recorded the highest LAI across all stages with values of 0.36, 3.01, 3.70 and 1.82 at 30, 60, 90 DAS and at harvest, respectively. These values were significantly higher compared to T_7 (absolute control), which recorded lower LAI of 0.30, 0.87, 1.10 and 0.57 at the respective stages. T_2 was significantly superior over T_5 [general recommended dose] (0.27, 2.24, 2.75 and 1.35 at 30, 60, 90 DAS and at harvest) and T_6 [soil fertility rating approach] (0.28, 2.32, 2.85 and 1.40 at 30, 60, 90 DAS and at harvest). Other STCR-based approaches, such as T_1 [STCR NPK (100 q ha^{-1})] and T_4 [STCR NPK + FYM (90 q ha^{-1})] were found to be on par with T_2 [STCR NPK + FYM (100 q ha^{-1})] at most stages but were slightly lower in LAI, particularly at 90 DAS and at harvest.

In HFS, the treatment T_2 [STCR NPK + FYM (100 q ha^{-1})] recorded the higher LAI across stages, with values of 0.37, 3.03, 3.76 and 1.88 at 30, 60, 90 DAS and at harvest, respectively. These values were significantly higher compared to the absolute control (T_7), which recorded the lowest LAI of 0.31, 1.01, 1.23 and 0.65 at 30, 60, 90 DAS and at harvest, respectively. T_2 [STCR NPK + FYM (100 q ha^{-1})] was significantly superior to T_5 [general recommended dose] (0.27, 2.27, 2.82 and 1.41 at 30, 60, 90 DAS and at harvest, respectively), T_3 [STCR NPK (90 q ha^{-1})] (0.29, 2.37, 2.94 and 1.47 at 30, 60, 90 DAS and at harvest, respectively) and T_6 [soil fertility rating approach] (0.28, 2.34, 2.90 and 1.45 at 30, 60, 90 DAS and at harvest, respectively) at all stages. Other STCR-based treatments like T_1 [STCR NPK (100 q ha^{-1})] and T_4 [STCR NPK + FYM (90 q ha^{-1})] performed on par with T_2 [STCR NPK + FYM (100 q ha^{-1})] at 30 and 60 DAS, but recorded slightly lower LAI at 90 DAS and at harvest.

In the farmer field of STZ, Shivamogga, T_2 [STCR NPK + FYM (100 q ha^{-1})] recorded significantly higher LAI values of 0.38, 2.98, 3.71 and 1.84 at 30, 60, 90 DAS and at harvest, respectively. These values were significantly superior to T_7 [absolute control], which recorded the lowest LAI values of 0.31, 0.84, 1.12 and 0.60 at 30, 60, 90 DAS and harvest, respectively. The performance of T_2 [STCR NPK + FYM (100 q ha^{-1})] was significantly higher than that of T_3 [STCR NPK (90 q ha^{-1})] (0.30, 2.31, 2.89 and 1.43), T_5 [general recommended dose] (0.29, 2.21, 2.76 and 1.37) and T_6 [soil fertility rating approach] (0.30, 2.29, 2.86 and 1.42) across all stages. The other

STCR-based treatments, T_1 [STCR NPK (100 q ha^{-1})] and T_4 [STCR NPK + FYM (90 q ha^{-1})], were statistically on par with T_2 at 30, 60, 90 DAS and at harvest.

The STCR NPK+FYM (100 q ha^{-1}) targeted yield demonstrated significantly improved growth parameters, such as plant height, number of leaves per plant, chlorophyll content and leaf area index. This enhancement can be attributed to the balanced nutrient supply from a combination of chemical fertilizers, organic sources and nano fertilizers. The initial nutrient demand was fulfilled by fertilizers, while organic matter gradually released nutrients upon decomposition. Thapar *et al*. (2019) observed a gradual increase in rice plant height under the STCR approach, attributing this growth to adequate NPK levels, which support the synthesis of nucleic acids essential for crop development. The study highlighted that the combined application of organic and inorganic fertilizers improved nutrient availability and uptake, thereby promoting better vegetative growth in plants. The sustained nutrient availability throughout the growth period enhanced nutrient uptake, thereby improving physiological processes and promoting better crop growth. These findings align with previous research by Verma *et al.* (2006), Karki and Ashok (2005) and Chandrakant (2015) in maize.

Use of nano fertilizers, due to their higher surface area and controlled release mechanisms, enhances nutrient use efficiency and promotes rapid cell division and elongation (Adhikari *et al*., 2021). This observation aligns with findings by Shah *et al*., (2022), who reported increased biomass accumulation and stem elongation in maize under nano-fertilizer-supplemented STCR strategies.

Similarly, the number of leaves per plant was significantly higher in STCR NPK+FYM (100 q ha^{-1} targeted yield), especially from 60 DAS onward. Leaf proliferation is directly linked to nitrogen availability and nano DAP used in the STCR approach likely facilitated enhanced nitrogen uptake, as also indicated by higher chlorophyll content and LAI. Goudar *et al*., (2020) highlighted the integrated use of STCR-based NPK with organics and nano fertilizers promoted vigorous vegetative growth, increasing leaf count and surface area.

Chlorophyll content is strongly correlated with nitrogen status in the leaf tissue (Yousaf *et al*., 2019). The sustained release of nitrogen from nano DAP under STCR ensured a steady supply of N during critical growth phases, resulting in prolonged greenness and delayed senescence (Krishna Murthy *et al*., 2025). Furthermore, elevated chlorophyll meter readings contributed to greater photosynthetic efficiency and biomass production (Sharma *et al*., 2023).

A well-developed LAI enhances light interception and canopy photosynthesis, crucial for yield maximization. The increase in LAI under nano DAP-aided STCR treatments aligns with the findings of Prasad *et al*. (2018), demonstrated that nano-formulated fertilizers improve LAI and crop productivity by reducing nutrient losses and optimizing nutrient availability in the rhizosphere.

In contrast, T_5 (General recommended dose), T_6 (soil fertility rating) and T_7 (absolute control) consistently showed lower values across all parameters, highlighting the inefficiency of blanket or conventional approaches compared to STCR, especially when enhanced with nano DAP and FYM. Notably, the T_4 [STCR NPK+FYM (90 q ha^{-1})] and T_1 [STCR NPK (100 q ha^{-1})] treatments also showed relatively higher performance but were statistically lower than T_2 [STCR NPK+ FYM (100 q ha^{-1})] during later growth stages, emphasizing the advantage of integrated nutrient management including nano inputs.

The results emphasize that STCR-based nutrient management along with nano DAP and FYM not only improves nutrient uptake and utilization but also significantly enhances maize growth and development. These findings are in line with recent studies promoting the synergy between targeted nutrient application and nanotechnology in sustainable crop production (Kumar *et al*., 2020; Singh *et al*., 2024).

TABLE 6

Influence of different approaches of nutrient application on yield parameters of maize

	Treatment details	LFS				MFS				HFS				Farmer field, STZ, Shivamogga			
		Cob length (cm)	No. of kernels per row	No. of rows per cob	Test weight (g)	Cob length (cm)	No. of kernels per row	No. of rows per cob	Test weight (g)	Cob length (cm)	No. of kernels per row	No. of rows per cob	Test weight (g)	Cob length (cm)	No. of kernels per row	No. of rows per cob	Test weight (g)
T_1	STCR NPK Targeted Yield 100 q ha^{-1}	17.92	39.83	17.20	31.33	18.83	39.92	18.40	32.33	18.67	40.00	19.20	33.00	18.80	40.00	18.40	32.67
T_2	STCR NPK + FYM Targeted Yield 100 q ha^{-1}	18.67	40.00	18.80	33.00	18.92	40.17	19.20	33.33	19.00	40.50	20.00	34.33	18.92	40.86	19.60	33.67
T_3	STCR NPK Targeted Yield 90 q ha^{-1}	18.00	38.83	16.40	30.33	18.00	39.00	17.60	31.33	18.08	39.33	18.40	32.33	17.50	39.17	17.60	31.33
T_4	STCR NPK + FYM Targeted Yield 90 q ha^{-1}	18.25	39.17	18.00	32.00	18.42	39.33	19.20	32.67	19.08	40.00	19.60	33.67	18.50	39.67	19.20	32.67
T_5	General Recommended dose	16.94	34.98	15.20	29.33	17.00	36.33	16.40	30.33	17.18	37.00	17.20	31.67	17.00	37.33	16.40	30.67
T_6	Soil Fertility Rating Approach	16.92	36.50	15.60	30.33	17.12	36.00	17.20	30.67	17.22	37.25	17.40	31.33	17.10	37.60	16.80	31.67
T_7	Absolute control	16.75	32.78	14.80	30.33	17.00	32.83	16.00	30.33	15.08	32.00	16.80	31.00	16.50	32.83	16.40	30.67
	S.Em. ±	0.60	1.78	0.58	0.35	0.62	1.78	0.61	0.45	0.62	1.79	0.64	0.80	0.62	1.80	0.62	0.45
	C.D. @ 5 %	1.71	5.06	1.65	NS	1.77	5.07	1.75	1.29	1.76	5.10	1.82	2.29	1.76	5.13	1.76	1.32

Influence of Various Approaches af Fertilizer Recommendation on Yield and Yield Attributes of Maize

The data pertaining to kernel yield (q ha^{-1}), stover yield (q ha^{-1}), cob length (cm), number of kernels per row, number of rows per cob and test weight (g) as influenced by different approaches of fertilizer recommendations is depicted in Table 6.

Kernel Yield and Stover Yield of Maize

Influence of different approaches of nutrient application on kernel yield and stover yield of maize is presented in Fig. 4. In low fertility strip, the treatment T_2 [STCR NPK + FYM (100 q ha^{-1})] recorded significantly higher kernel yield (97.09 q ha^{-1}) and stover yield (137.87 q ha^{-1}) compared to general recommended dose (T_5: 82.32 q ha^{-1} and 116.90 q ha^{-1}, respectively), soil fertility rating approach (T_6: 83.79 q ha^{-1} and 118.98 q ha^{-1}, respectively), STCR NPK (90 q ha^{-1}) (T_3: 85.31 and 121.14 q ha^{-1}) and absolute control (T_7: 47.91 q ha^{-1} and 68.04 q ha^{-1}, respectively). Other STCR-based nutrient recommendation approaches like STCR NPK (100 q ha^{-1}) (T_1: 94.91 and 134.77 q ha^{-1}) and STCR NPK+FYM (90 q ha^{-1}) (T_4: 90.00 and 127.80 q ha^{-1}) were statistically on par with T_2 [STCR NPK + FYM (100 q ha^{-1})] but showed numerically lower yield.

In the medium fertility strip, significantly higher kernel yield (98.05 q ha^{-1}) and stover yield (141.38 q ha^{-1}) was observed under T_2 [STCR NPK + FYM for a target yield of 100 q ha^{-1}], compared to T_5 [general recommended dose] (83.30 and 120.12 q ha^{-1}), T_6 [soil fertility rating approach] (84.77 and 122.23 q ha^{-1}), T_3 [STCR NPK for 90 q ha^{-1}] (86.27 and 124.40 q ha^{-1}) and T_7 [absolute control] (49.18 and 70.92 q ha^{-1}), all of which recorded significantly lower values. Among the STCR treatments, T_1 [STCR NPK (100 q ha^{-1})] and T_4 [STCR NPK + FYM (90 q ha^{-1})] produced yields of 95.87 and 138.24 q ha^{-1} and 90.96 and 131.16 q ha^{-1}, respectively, which were statistically comparable with T_2 [STCR NPK + FYM (100 q ha^{-1})], though slightly lower in yield.

In high fertility strip, the trend continued with T_2 [STCR NPK + FYM for 100 q ha^{-1}] producing the maximum kernel yield (98.98 q ha^{-1}) and stover yield (144.51 q ha^{-1}), which were significantly higher than the yields under T_5 [general recommended dose] (84.24 and 122.99 q ha^{-1}), T_6 [soil fertility rating approach] (85.70 and 125.12 q ha^{-1}), and T_7 [absolute control] (50.11 and 73.17 q ha^{-1}). Among the STCR-based treatments, T_1 [STCR NPK (100 q ha^{-1})] (96.80 and 141.33 q ha^{-1}), T_3 [STCR NPK for 90 q ha^{-1}] (87.20 and 127.32 q ha^{-1}) and T_4 [STCR NPK + FYM (90 q ha^{-1})] (91.89 and 134.16 q ha^{-1}) were on par with T_2 [STCR NPK + FYM for 100 q ha^{-1}] but numerically lower.

In the southern transition zone (Shivamogga), the treatment T_2 [STCR NPK + FYM (100 q ha^{-1})] recorded the highest kernel yield (97.82 q ha^{-1}) and stover yield (145.75 q ha^{-1}), which was significantly superior to T_5 [general recommended dose] (82.95 and 122.06 q ha^{-1}), T_6 [soil fertility rating approach] (84.67 and 125.23 q ha^{-1}) and T_7 [absolute control] (48.58 and 74.01 q ha^{-1}). Other STCR-based treatments, including T_1 [STCR NPK for 100 q ha^{-1}] (95.77 and 142.26 q ha^{-1}), T_3 [STCR NPK (90 q ha^{-1})] (86.15 and 129.90 q ha^{-1}) and T_4 [STCR NPK + FYM (90 q ha^{-1})] (91.06 and 134.02 q ha^{-1}), were found to be statistically on par with T_2 [STCR NPK + FYM (100 q ha^{-1})], though T_2 [STCR NPK + FYM (100 q ha^{-1})] consistently recorded numerically higher yields. This result highlights the efficacy of the STCR approach, particularly when integrated with FYM.

Cob Length

The yield parameters data is presented in Table 6. The cob length of maize was significantly influenced by different fertilizer management approaches across all fertility strips. The treatment T_2 [STCR NPK + FYM (100 q ha^{-1})] consistently recorded the longest cob length in all three fertility strips-18.67 cm in LFS, 18.92 cm in MFS and 19.00 cm in HFS, which was significantly superior to the general recommended dose (T_5: 16.94, 17.00 and 17.18 cm, in LFS, MFS and HFS, respectively), soil fertility rating approach

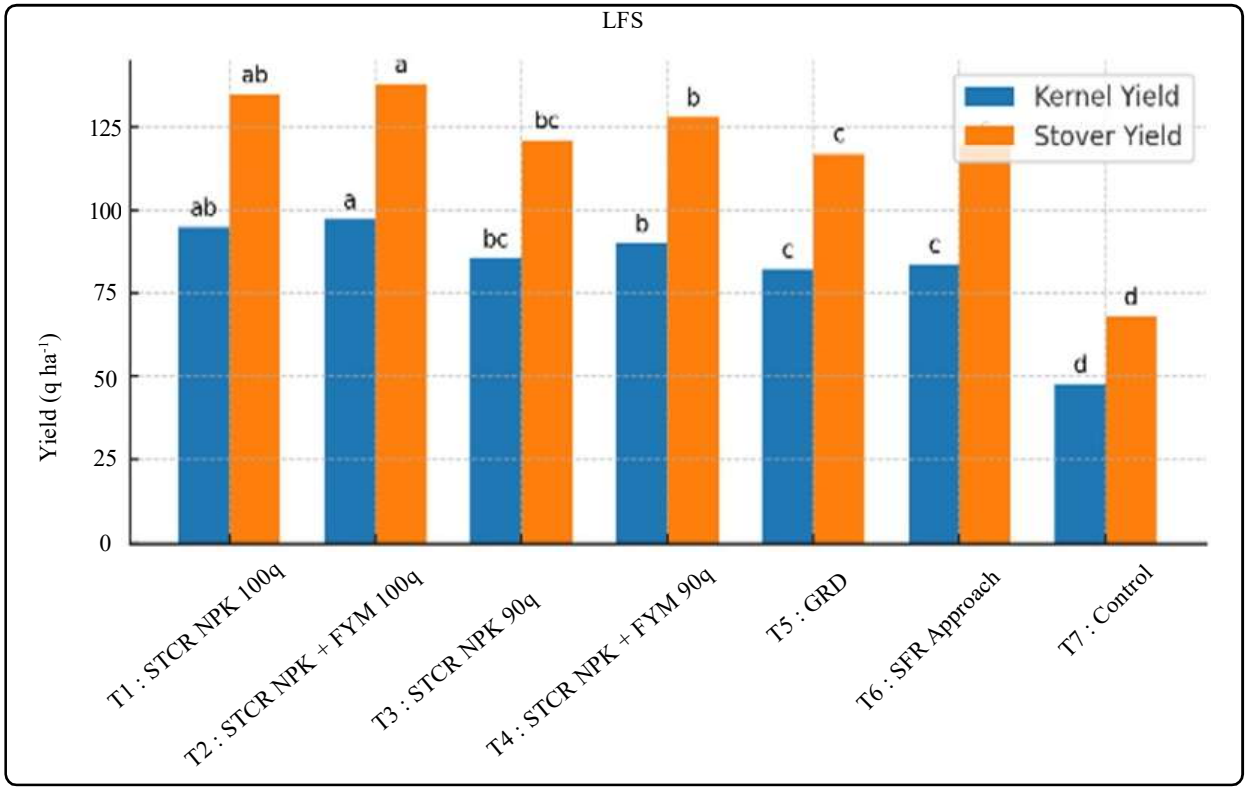
LFS
Kernel Yield
Stover Yield
Yield (q ha-1)
0
25
50
75
100
125
ab
a
bc
b
c
c
d
T1 : STCR NPK 100q
T2 : STCR NPK + FYM 100q
T3 : STCR NPK 90q
T4 : STCR NPK + FYM 90q
T5 : GRD
T6 : SFR Approach
T7 : Control

Fig. 4 (a)

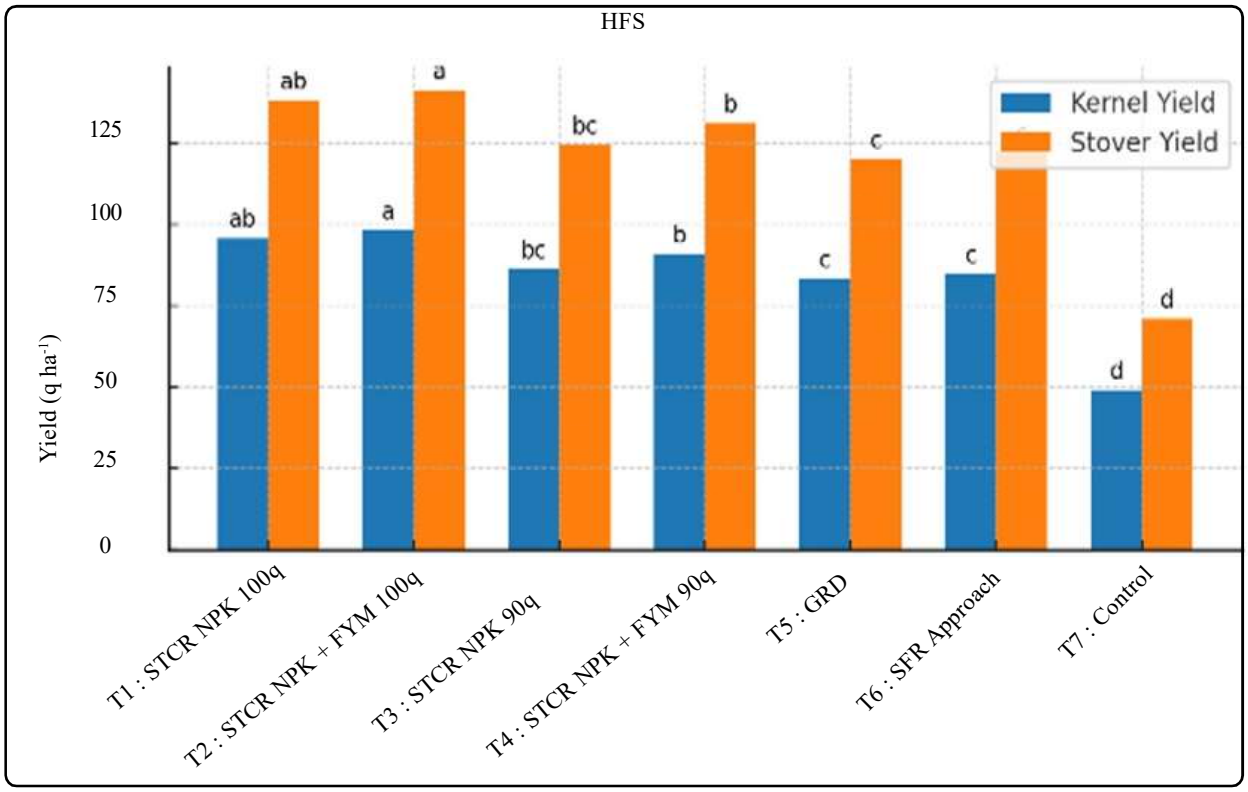
HFS
Kernel Yield
Stover Yield
Yield (q ha-1)
0
25
50
75
100
125
ab
a
bc
b
c
c
d
T1 : STCR NPK 100q
T2 : STCR NPK + FYM 100q
T3 : STCR NPK 90q
T4 : STCR NPK + FYM 90q
T5 : GRD
T6 : SFR Approach
T7 : Control

Fig. 4 (b)

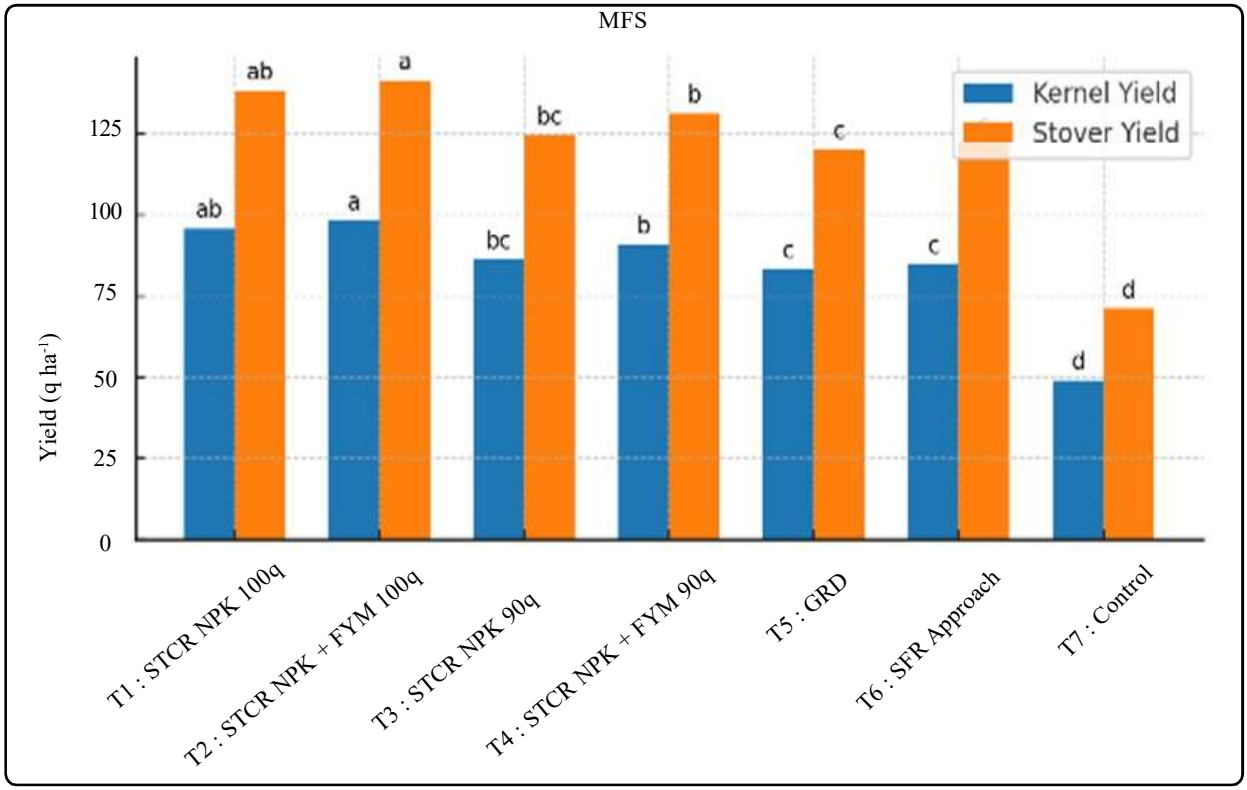

Fig. 4 (c)

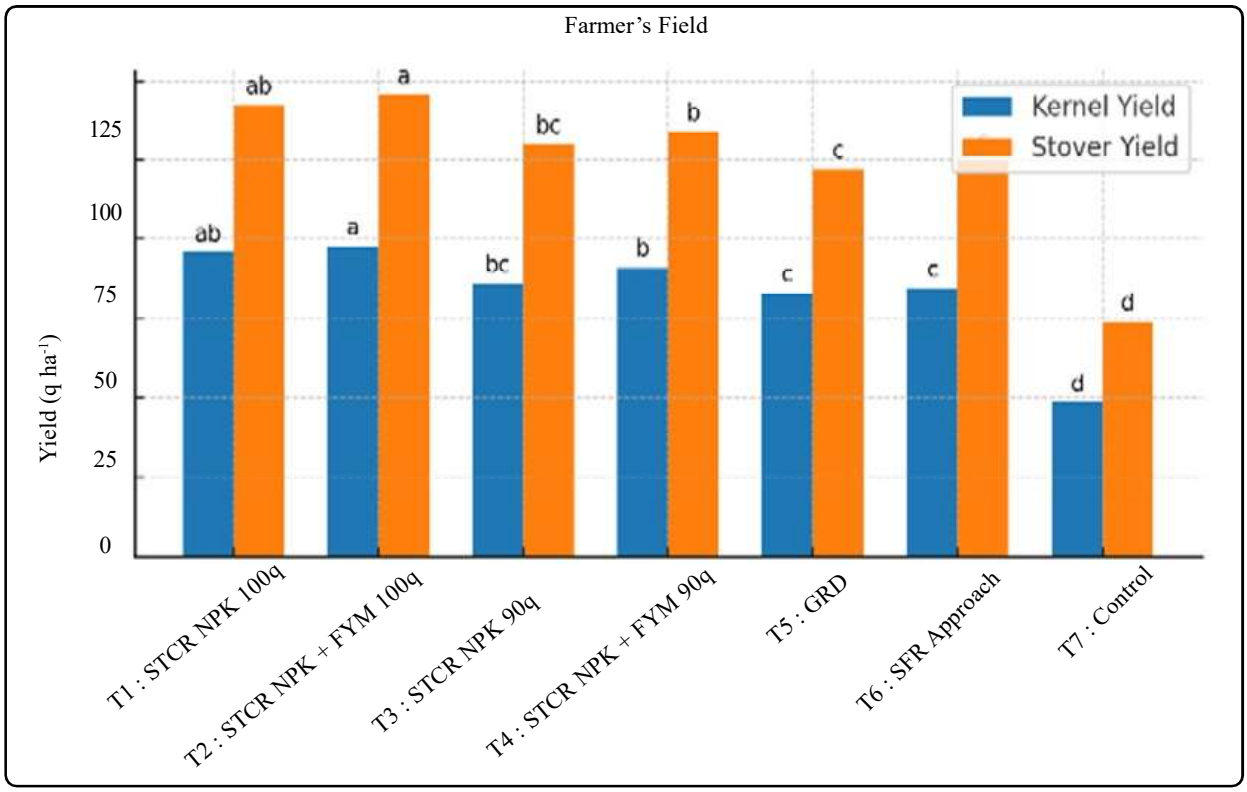

Fig. 4 (d)

Fig. 4: Influence of different approaches of nutrient application on kernel yield and stover yield of maize

(T_6: 16.92, 17.12 and 17.22 cm, in LFS, MFS and HFS, respectively) and absolute control (T_7: 16.75, 17.00 and 15.08 cm, in LFS, MFS and HFS, respectively). Other STCR-based nutrient recommendations such as T_1 [STCR NPK for 100 q ha^{-1}], T_3 [STCR NPK for 90 q ha^{-1}] and T_4 [STCR NPK + FYM for 90 q ha^{-1}] were statistically on par with T_2 [STCR NPK + FYM (100 q ha^{-1})] across all strips but recorded slightly lower cob length values. The mean values of cob length, farmers field of Shivamogga (STZ) also confirmed this trend, with T_2 [STCR NPK + FYM (100 q ha^{-1})] registering the highest average cob length (18.92 cm), followed closely by T_1 [STCR NPK for 100 q ha^{-1}] (18.80 cm) and T_4 [STCR NPK + FYM for 90 q ha^{-1}] (18.50 cm), all of which outperformed T_5 [general recommended dose] (17.00 cm), T_6 [soil fertility rating approach] (17.10 cm) and T_7 [absolute control] (16.50 cm) significantly.

Number of kernels per row

The number of kernels per row in maize was notably enhanced by the application of integrated nutrient management practices (Table 6). Across all fertility strips-LFS, MFS and HFS-the treatment T_2 [STCR NPK + FYM for 100 q ha^{-1}] consistently registered the higher number of kernels per row, with 40.00, 40.17 and 40.50, in LFS, MFS and HFS, respectively. A significantly greater value was observed in comparison with the absolute control (T_7: 32.78, 32.83 and 32.00 in LFS, MFS and HFS, respectively).

Although T_1 [STCR NPK for 100 q ha^{-1}] (39.83, 39.92 and 40.00 in LFS, MFS and HFS, respectively), T_3 [STCR NPK for 90 q ha^{-1}] (38.83, 39.00 and 39.33 in LFS, MFS and HFS, respectively), general recommended dose (T_5: 34.98, 36.33 and 37.00 in LFS, MFS and HFS, respectively), soil fertility rating approach (T_6: 36.50, 36.00 and 37.25 in LFS, MFS and HFS, respectively) and T_4 [STCR NPK + FYM for 90 q ha^{-1}] (39.17, 39.33 and 40.00 in LFS, MFS and HFS, respectively) improved kernel numbers compared to T_7 [absolute control] their showed a marginal decline compared to T_2 [STCR NPK + FYM for 100 q ha^{-1}] and statistically comparable. The mean values across Shivamogga revealed a similar trend, with T_2 [STCR NPK + FYM for 100 q ha^{-1}] achieving significantly maximum number of kernels per row (40.86) compared to T_7 [absolute control] (32.83), however T_2 [STCR NPK + FYM for 100 q ha^{-1}] found on par with T_1 [STCR NPK for 100 q ha^{-1}] (40.00), T_3 [STCR NPK for 90 q ha^{-1}], T_4 [STCR NPK + FYM for 90 q ha^{-1}] (39.67), T_5 [general recommended dose] (37.33), T_6 [soil fertility rating approach] (37.60).

Number of rows per cob

Application of STCR-based nutrient management practices had a positive effect on the number of rows per cob in maize (Table 6). In the low fertility strip**,** the maximum number of rows per cob was observed in the treatment T_2 [STCR NPK + FYM for 100 q ha^{-1}], recording 18.80, found to be on par with T_4 [STCR NPK + FYM for 90 q ha^{-1}] (18.00) and T_1 [STCR NPK for 100 q ha^{-1}] (17.20). The recorded values were markedly higher than those in T_3 [STCR NPK (90 q ha^{-1})] (16.40), T_5 [general recommended dose] (15.20), T_6 [soil fertility rating approach] (15.60) and T_7 [absolute control] (14.80).

In the medium fertility strip, T_2 [STCR NPK + FYM for 100 q ha^{-1}] (19.20) showed comparable results with T_4 [STCR NPK + FYM for 90 q ha^{-1}] (19.20), T_3 [STCR NPK (90 q ha^{-1})] (17.60) and T_1 [STCR NPK for 100 q ha^{-1}] (18.40). Significant reduction in number of rows per cob was noticed in T_5 [general recommended dose] (16.40), T_6 [soil fertility rating approach] (17.20) and in absolute control (T_7) recorded 16.00, indicating a significant reduction in the absence of nutrient application. In the high fertility strip, T_2 [STCR NPK + FYM for 100 q ha^{-1}] showed the highest number of rows per cob (20.00), which was statistically comparable with T_4 [STCR NPK + FYM for 90 q ha^{-1}] (19.60), T_1 [STCR NPK for 100 q ha^{-1}] (19.20) and T_3 [STCR NPK for 90 q ha^{-1}] (18.40). In contrast, reduced values were noticed under T_5 [general recommended dose] (17.20), T_6 [soil fertility rating approach] (17.40) and absolute control (T_7), which recorded 16.80 rows per cob.

In the farmers field of STZ, Shivamogga, the treatment T_2 [STCR NPK + FYM for the targeted yield of 100 q ha^{-1}] recorded the higher number of rows per cob (19.60), which was statistically on par with T_4 [STCR NPK + FYM for the targeted yield of 90 q ha^{-1}] (19.20) and T_1 [STCR NPK for the targeted yield of 100 q ha^{-1}] (18.40). The recorded values were considerably higher than those observed in T_3 [STCR NPK for the targeted yield of 90 q ha^{-1}] (17.60), T_5 [general recommended dose] (16.40), T_6 [soil fertility rating approach] (16.80) and T_7 [absolute control], which recorded the lowest number of rows per cob (16.40).

Test Weight

Maize kernel test weight responded distinctly to nutrient management approaches across varying soil fertility gradients (Table 6). In the low fertility strip, although numerical differences were observed, the variation in test weight across treatments was statistically non-significant, with T_2 [STCR NPK + FYM for targeted yield of 100 q ha^{-1}] recording 33.00 g, slightly ahead of T_4 [STCR NPK + FYM for the targeted yield of 90 q ha^{-1}] (32.00 g), T_1 [STCR NPK for the targeted yield of 100 q ha^{-1}] (31.33 g) and others like T_3 [STCR NPK for the targeted yield of 90 q ha^{-1}] (30.33 g), T_5 [general recommended dose] (29.33 g), T_6 [soil fertility rating approach] and T_7 [absolute control] (30.33 g each).

In medium fertility soils, the T_2 [STCR NPK + FYM for targeted yield of 100 q ha^{-1}] treatment again produced the higher test weight (33.33 g), significantly outperforming conventional practices such as the general recommended dose (30.33 g), soil fertility rating approach (30.67 g) and absolute control (30.33 g) and T_3 [STCR NPK (90 q ha^{-1})] (31.33). Other STCR-based nutrient management practices like T_1 [STCR NPK for the targeted yield of 100 q ha^{-1}] (32.33 g) and T_4 [STCR NPK + FYM for the targeted yield of 90 q ha^{-1}] (32.67 g) performed on par with T_2 [STCR NPK + FYM for targeted yield of 100 q ha^{-1}] but recorded slightly lower values.

In the high fertility strip, where T_2 [STCR NPK + FYM (100 q ha^{-1})] showed the higher test weight of 34.33 g, significantly superior over T_5 [general recommended dose] (31.67 g), T_6 [soil fertility rating approach] (31.33 g) and T_7 [absolute control] (31.00 g). All other treatments showed significantly better performance compared to the conventional nutrient recommendation method. In the farmers field of STZ, Shivamogga, the T_2 [STCR NPK + FYM for targeted yield of 100 q ha^{-1}] treatment recorded the higher test weight (33.67 g), significantly higher than the conventional practices such as the general recommended dose (30.67 g), soil fertility rating approach (31.67 g), absolute control (30.67 g) and T_3 [STCR NPK for 90 q ha^{-1}] (31.33 g). Other STCR-based nutrient management treatments like T_1 [STCR NPK for 100 q ha^{-1}] and T_4 [STCR NPK + FYM for 90 q ha^{-1}], which both recorded 32.67 g, were statistically on par with T_2 but showed marginally lower test weight values.

The results on yield and yield attributes revealed that the STCR-based fertilizer recommendation, both with NPK alone and NPK combined with FYM, was superior to the LMH and soil fertility rating approaches for both yield targets. The study highlights that site-specific nutrient management through the STCR approach, particularly when integrated with organic sources like FYM and emerging inputs such as nano-fertilizers (Nano DAP), markedly enhanced maize yield and yield components under different fertility gradients. The higher productivity observed under STCR treatments may be attributed to the targeted yield strategy, which ensures efficient fulfilment of crop nutrient requirements. These findings are in agreement with Isha *et al.* (2024), who reported that nitrogen application during the initial growth stages stimulates vegetative development, favourably influencing yield potential through its key role in chlorophyll formation and protein synthesis. Phosphorus was equally important, supporting energy transfer, metabolic processes and seed development, thereby contributing to increased yield. Potassium application brought both quantitative and qualitative improvements in maize performance. The significantly higher kernel and stover yields

obtained under the STCR NPK + FYM treatment for the 100 q ha^{-1} target could be ascribed to the balanced nutrient supply adjusted to crop demand and soil fertility status. Moreover, the incorporation of FYM improved soil structure, organic matter and microbial activity, leading to enhanced nutrient release and uptake (Ramesh *et al*., 2017). The superiority of STCR-based recommendations over conventional fertilizer doses and fertility rating approaches is consistent with the findings of Singh *et al.* (2020), who emphasized that STCR-guided nutrient management enhances nutrient use efficiency while sustaining crop productivity.

The higher yields achieved under the NPK + FYM approach at elevated yield targets, compared to NPK alone, can be attributed to the addition of FYM, which enhanced microbial activity and supplied sufficient carbon, thereby improving nutrient availability and contributing to greater crop growth and productivity (Abhirami *et al*., 2023). Although lower quantities of phosphorus fertilizers were applied in the STCR-based treatments relative to GRD and SFR, yields were significantly higher in the STCR plots. This highlights the efficient utilization of native soil phosphorus, suggesting that phosphorus fertilizer costs can be minimized when soils possess adequate available phosphorus.

The superior nutrient uptake and higher nutrient use efficiency achieved through the STCR approach, as compared to SFR and GRD, had a favourable impact on growth and yield attributes, thereby contributing to increased maize yield. In addition, the synergistic effect of combining organic and inorganic sources under the STCR NPK+FYM treatment likely improved the chemical, physical and biological properties of the soil, which in turn supported higher productivity (Spoorthishankar *et al*., 2025). These results are consistent with the observations of Raghav *et al.* (2019), who reported that enhanced yield attributes of soybean under the STCR approach, relative to SFR, GRD and farmer's practice, were due to the balanced nutrient application based on soil test values and the crop's nutrient requirement for achieving a predetermined yield target.

Moreover, recent research supports the incorporation of nano-fertilizers, such as nano DAP, which offer controlled nutrient release, reduced nutrient losses and improved crop response at lower application rates (Subramanian *et al*., 2021).

Cob length and the number of kernels per row are the direct indicators of sink capacity and overall plant health. Treatments under STCR + FYM not only ensured sufficient nutrient supply throughout the critical growth stages but also improved reproductive development, which led to significantly superior cob dimensions. These findings are in agreement with Meena *et al.* (2018), who observed that integrated nutrient management enhanced cob size and kernel number owing to improved nitrogen availability and efficient nutrient allocation. The greater number of rows per cob under the STCR-based fertilizer recommendation, especially with the inclusion of FYM, highlights the beneficial role of balanced and continuous nutrient supply in promoting floral development and improving cob architecture. The presence of organic inputs may have contributed to enhanced soil biological activity and better nutrient synchrony during tasseling and silking stages, which are critical for cob formation (Kumar *et al*., 2019).

Test weight, an important grain quality trait, was also maximized under the STCR NPK + FYM (100 q ha^{-1}) treatment. Adequate nutrient availability during the grain filling stage, especially nitrogen and phosphorus, is known to improve starch accumulation and kernel density. Integration with FYM may have prolonged nutrient release, while the potential addition of nano-nutrients like nano DAP could further aid in macronutrient sufficiency and physiological efficiency during critical stages (Mahajan, 2022).

This study shows that site-specific targeted fertilizer recommendations, when integrated with farmyard manure and nano-DAP substantially enhance maize growth, physiology, yield and yield components across fertility gradients and farmers fields in the southern transition zone (Shivamogga). The STCR NPK + FYM treatment for a 100 q ha^{-1} target (T_2) consistently recorded the highest plant height, leaf number, chlorophyll meter readings, leaf area index,

cob length, kernel attributes and both kernel and stover yields and was statistically superior to conventional approaches (general recommended dose and soil fertility rating) and absolute control. Other STCR variants (T_1, T_3, T_4) performed comparably but generally produced numerically lower values than T_2, highlighting the added benefit of combined organic-inorganic nutrient supply and nano-fertilizer aided nutrient synchrony.

The results imply that balancing nutrient supply to crop demand and supplementing with FYM improves soil physical, chemical and biological functioning, enhances nutrient use efficiency and supports sustained nutrient availability during critical reproductive stages-thereby increasing sink capacity and grain filling. Use of nano-DAP likely contributed to improved early nutrient availability and sustained N supply, explaining higher chlorophyll and LAI values. Practically, adoption of STCR + FYM offers a pathway to raise maize productivity while potentially reducing P fertilizer requirement.

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Phage Display for Antibody Development against Recombinant SAP11 Effector Protein of Phytoplasma

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Abstract

Phytoplasma are obligate intracellular parasites that cause devastating diseases with serious economic or environmental impacts. The present research focused on developing antibodies against one of the key effector proteins (SAP11) of AYWB phytoplasma. Initially, the recombinant SAP11 was produced at large scale using bacterial expression system and resultant was purified through Ni-NTA affinity chromatography. The purified SAP11 protein was then used as an antigen for antibody development using phage display technology. In this approach, Tomlinson's phage libraries were amplified using M13K07 helper phages and the phages displaying scFv clones specific to SAP11 were selected through multiple rounds of biopanning. Four rounds of biopanning was performed and the population of phage from each round were evaluated for its binding ability against SAP11 by polyclonal ELISA. The results revealed that the phages from the fourth round of biopanning exhibited slightly higher affinity than the third round and further these phages were used to generate monoclonal antibodies. In first monoclonal screening, the 40 colonies were screened by ELISA and shortlisted four scFv clones. However, selected clones failed to amplify the scFv genes and did not release the expected inserts upon digestion with *Sfi*I and *Not*I enzymes. In second monoclonal screening, colony PCR was performed for 288 colonies prior to ELISA, only 16 colonies (approximately 5%) showed successful amplification. The low percentage of positive clones may indicate the inefficiencies in the biopanning process or low affinity of scFv clones towards the target antigen. Despite few positive results in ELISA, nofunctional scFv gene was conclusively identified. The part of research reported the challenges encountered during monoclonal antibody development and key trouble shooting ideas that may benefit future researchers in this area. Overall, the study successfully generated the polyclonal antibodies against SAP11 protein, which hold potential for detecting phytoplasma pathogens in field samples.

Keywords : SAP11 protein, Ni-NTA affinity purification, Phage display, Biopanning, ELISA

Phytoplasma are a group of organisms that lack a cell wall, pleomorphic in shape, with a single membrane, 200-800 nm in diameter and possess a very small genome of about 680-1530 kb (Doi *et al.,* 1967 and Lee *et al.,* 2000). Phytoplasmas are plant pathogenic bacteria associated with devastating damage to over 1000 plant species worldwide including agricultural, horticultural, ornamental, trees and weed species (Bertaccini and Lee, 2018) and causes significant losses in various parts of the world. In India, phytoplasma diseases have been reported for almost 150 years, starting with coconut root (wilt) in Kerala (1874). Subsequent report includes sandal spike in Karnataka, yellow leaf disease of arecanut, little leaf of brinjal, sesame phyllody and sugarcane grassy shoot. Recently, Muttappagol *et al.* (2022)

reported the association of 16SrI-D group of phytoplasma with phyllody disease of niger (*Guizotia abyssinica* L.). Additionally, phytoplasma infection have also been characterized in weed species such as Datura and Parthenium species (Bandakkanavar *et al.,* 2021). Since then, more than 172 plant species have been found as hosts of ten phytoplasma groups (Rao, 2021). In India the limited studies have been performed with losses caused by phytoplasmas infection in crops like brinjal, sesame, sugarcane, arecanut and coconut palms. Reported studies include 10-15 per cent yield loss from chickpea phyllody (Ghanekar *et al.,* 1988), up to 40 per cent of yield loss due to sugarcane grassy shoot (Viswanathan, 2001) and 60 per cent yield loss due to sesame phyllody (Rao *et al.,* 2015).

Research on phytoplasma remains limited because of their recalcitrant biological properties like confinement to phloem tissue, non-transmissibility by manual inoculation, rigidity in vector specificity and inability to culture in synthetic media which altogether made them as the least defined plant pathogens (Maejima *et al.,* 2014). Additionally, phytoplasma are transmitted through insect vectors and measures concerning vectors could reduce disease incidence, however complete elimination of vectors is not achievable.

Phytoplasmas in plant and insect tissues are generally detected by electron microscopy, staining with DNA-binding dyes in conjunction with light and fluorescent microscopy, serological methods, DNA-DNA hybridisation and polymerase chain reaction (PCR) techniques (Rajeev *et al.,* 2011). The serologic detection and identification using specific antiserum is an economical and convenient method that allows analysis of many samples within a short time period (Wambua *et al.,* 2017). Antibodies are essential component in serological based detection and can be developed as a polyclonal or monoclonal. The monoclonal antibodies are specifically designed to target single epitope of an antigen and can be developed using phage display or hybridoma technology. Phage display technology was first described by George P. Smith in 1985, when he demonstrated the display of peptides on filamentous phage by fusing the peptide of interest to gene III of filamentous phage (Smith, 1985). This technology confers the potential for generating antibodies from universal libraries against any conceivable molecule of sufficient size and omits the limitations of the immune system (Roth *et al.,* 2021). Antibody libraries are made up of recombinant phage particles, engineered to express the antibody genes fused to pIII coat protein, enabling display of the antibodies outside the phage surface (Roth *et al.,* 2021). In plants, the antibody phage display is mainly applied in the field of disease diagnosis, to name a few Raeisi *et al.* (2020) used phage display to develop scFv antibodies to detect *chickpea chloratic dwarf virus* (CpCDV), Kumar *et al.* (2016) produced scFv antibodies against *Sclerotium rolfsii* lectin (SRL) using phage display technology and similarly Yang *et al.* (2013) generated scFv against HrpA of *Pseudomonas syringae* pv. tomato.

The immuno dominant membrane proteins (IMPs) are most commonly used antigens among the phytoplasma proteins for the production of specific antibodies (Loi *et al.,* 2002 and Shahryari *et al.,* 2010). IMPs are present in large numbers in the phytoplasma membrane and represent promising targets for the development of detection assays. Polyclonal antisera and monoclonal antibodies that are raised against IMPs have been successfully used for the detection and differentiation of several phytoplasma diseases, such as European stone fruit yellows (Mergenthaler *et al.,* 2001), apple proliferation phytoplasma (Loi *et al.,* 2002), Witches' broom of lime (Shahryari *et al.,* 2010) and grapevine yellows (flaves cencedoree) (Filippin *et al.,* 2019).

The present study involves development of antibodies against one of the effector proteins of aster yellow phytoplasma (AY-WB). AY-WB infects dicots and secretes SAP11, an effector that causes shoot proliferation, leaf deformities, weakened defense responses, phosphate starvation, altered volatile compound synthesis and delayed flowering (Sugio *et al.,* 2011; Lu *et al.,* 2014 and Tan *et al.,* 2016). SAP11 homologs are identified in diverse

class of phytoplasma like *Candidatus* Phyto plasmamali, Peanut witches' broom phytoplasma and OY-M phytoplasma that are phylogenetically distant. Importantly, the SAP11 effector from all these phytoplasma strains found to be highly conserved in function (Chang *et al.,* 2018).

The effector molecule can serve as a better target antigen, because the phytoplasma membrane proteins are confined to the phloem sieve cells, while the effector molecules are released out of the phytoplasma and travel all parts of the plant. The phytoplasma titre is one of the factors which affects the sensitivity of detection. Hence, for detection purpose the leaf sample is sufficient for analysis using effectors as target antigen, whereas to detect IMPs, the sample must contain plenty of phloem cells. This may exhibit challenges in case of hardy crops and we hypothesize that using effectors as a target antigen enhances the sensitivity of the detection.

The present study deals with the development of antibodies against one of the effector proteins of phytoplasma (SAP11) using the phage display technique which can be later applied in the field of diagnostics to detect phytoplasma infection.

Material and Methods

Preparation of Antigen

The SAP11 effector protein from AYWB phytoplasma was used as antigen. The recombinant *E. coli* BL21 harboring pET28a+SAP11 was used for protein expression, for which it was cultured and induced using 1mM of IPTG. Following induction, the crude proteins were isolated and purified using Ni-NTA affinity chromatography column. The eluted protein was then dialyzed with 1X PBS. The purity and expression of the SAP11 protein was confirmed by SDS-PAGE and its concentration was determined using standard Bradford method.

Isolation of M13 K07 Bacteriophage

M13 K07are helper phages used to amplify the secondary phages that lack functional lytic cycle. The *E. coli* K12 strains were used as host to propagate these helper phages. Phage isolation and purification was done using PEG-NaCl precipitation method (Chandrashekara, 2006). The standard spot test and double agar layer (DAL) method was used to validate the phage activity and further, the phage yield was determined using both plaque assay and spectrophotometric absorbance- based measurement.

Plaque Count Method

$$\text{Virions/ml} = \frac{\text{Total number of plaques per plate X 100 X dilution factor}}{\text{Number of ml used for infection}}$$

Absorption Spectroscopic Method

$$\text{Virions/ml} = \frac{(A269 - A320) \times 6 \times 10^{16}}{\text{No. of bases/phage}}$$

No. of bases/phages = 6400 for M13 phage

No. of bases/phages = 8500 for scFv

Amplification of Tomlinson's Phage Library

Tomlinson's phage libraries were procured from Dr. Narayan Moger, Institute of Agri Biotechnology, University of Agricultural Sciences, Dharwad. The aliquot of bacterial library (1 ml) stock containing about $1x10^{10}$ clones was used for amplification of the scFv phagemids. Amplification, isolation and purification was done according to standard protocol (Chandrashekara, 2006). The obtained phage supernatant got filtered through 0.45 mm filter and stored at 4 °C in presence of 3 per cent BSA made in 1XPBS.

Selection of Phages Carrying Antibody Specific to SAP11 Protein

First Round of Biopanning (BP)

The phages carrying antibody specific to SAP11 can be selected using immunotube by a process called biopanning. The first round of biopanning was initiated by coating the prepared antigen (SAP11), a concentration of 200 µg in 4 ml of coating buffer on

to the NUNC™ immunotube and incubated overnight at room temperature. Next day, the tube was washed thrice with 1X PBS. 3 per cent BSA made in 1X PBS was added to the immunotube and incubated at 37 °C for 2 hour to block. The tube was then washed thrice with 1X PBS. 10^{12} phages isolated from scFv Tomlinson's phage libraries (4 mL) was added to the tube and incubated for 30 minutes at room temperature rotating continuously on an under-and-over turnable shaker and then kept still for further 90 minutes at room temperature. The tube was washed 10 times with 1X PBS containing 0.05 per cent tween-20 then 10 times with 1X PBS to remove detergent. The bound phages were eluted by adding 1ml of 100mM triethylamine and kept in rotating shaker continuously for 10 minutes. Then the phages were quick neutralized by adding 0.5 ml of 1M Tris, pH 7.4. After first elution, another 200 µl of 1M Tris, pH 7.4 was added to the immunotube to neutralize the remaining phage in the tube.

Then the eluted phages were amplified by plated in a selection media as mentioned. Briefly, 50 ml tube containing 9.25 ml of an exponentially growing culture of K12 was taken and the 0.75 ml of the eluted phages was added. The culture was incubated in a water bath for 30 minutes at 37 °C without shaking. Then, infected K12 culture was spun at 3,300 g for 10 minutes to pellet the cells, which was suspended in 1ml of 2x Tryptone-Yeast extract (TYE). 1 ml K12 infected cells from eluted phages were plated on three big petri plates with TYE containing 100 µg/ml carbenicillin, 2 µg/ml tetracycline and 1 per cent glucose. The plates were incubated at 37 °C overnight.

Further Rounds of Selection

In order to isolate antibody having higher affinity towards the target antigen, the biopanning process was repeated 2-3 rounds, 2-3 ml of 2xTYE, 15 per cent glycol mixture was added to the bacterial colonies which were grown on the big petri dishes and the cells were loosened with a glass spreader. 100 µl of the above scraped bacteria were added to 100 ml of 2xTYE containing 100 µg/ml carbenicillin, 2 µg/ml tetracycline and 1 per cent glucose and the remaining bacteria were stored at -80 °C. Bacteria were grown with shaking at 37 °C until it reached OD (600 nm) of 0.5. Ten ml of this bacterial culture was taken separately in clean tubes and infected with M13 K07 helper phage in the ratio of 1:20 (bacterial cells: helper phage). The culture tube was incubated without shaking in 37 °C water bath for 30 minutes. The infected cells were spun at 3,300 g for 10 minutes at 10 °C, resuspended the pellet gently in 100 ml of 2xTYE containing 100 µg/ml carbenicillin, 2 µg/ml tetracycline and 25 µg/ml kanamycin and incubated at 37 °C overnight. The overnight grown culture was taken and spun at 3,300 g for 30 minutes. To the supernatant, 1/5 volume (20 ml) PEG-NaCl was added mixed well and kept on ice for 1 hour. Further, the supernatant was spun at 3,300g for 30 minutes and then aspirated off the supernatant. The phage pellet was resuspended in 2ml 1X PBS and spun at 10,000rpm for 10 minutes in a micro centrifuge to remove bacterial debris. One ml of this phage was used for next round of biopanning and remaining 1 ml was stored at 4 °C.

In order to rule out the scFv phages which were bound to the blocking reagents, during each round of biopanning the blocking buffers were changed. Hence, the second round of biopanning was performed similar to first round, except that in second round, the immunotube was blocked with 3 per cent skim milk instead of BSA. Following the binding of the phages from the first round of BP, the immunotube was washed 20 times with 1X PBS containing 0.05 per cent tween-20 followed by 20 additional washes with 1XPBS. The third round of biopanning was done similar to the second round, but the blocking reagent was changed to 3 per cent casein. During fourth round of biopanning the tubes were blocked with 3 per cent BSA as a blocking reagent instead of casein.

Screening of Phage Particles by ELISA

A population of phage produced at each rounds of selection was screened for its binding ability to the selected antigen by ELISA.

Dot Blot ELISA

Initially, the dot blot assay was performed according to the protocol described by Mishra *et al.* (2022).

Briefly, 10 µL of SAP11 antigen with different dilution was dotted on to nitrocellulose membrane, air dried and subsequently blocked with 3 per cent Bovine serum albumin for 30 mins. The membrane was then washed thrice with interval of 10 minutes with 1X PBS and derived phages (serving as the primary antibody) were added at concentration $1x10^{10}$ and incubated for 1 hour. Then the membrane was washed thrice with 1X PBS with interval of 10 minutes. An anti-M13 HRP-conjugated secondary antibody was then applied and incubated for 2 hours. After a final set of washes, color development was performed using TMB substrate for 30 minutes in the dark.

Polyclonal ELISA

The purified SAP11 antigen was serially diluted in carbonate buffer starting from 2.5 µg/mL to 0.156 µg/mL and 200 µL each were coated onto the ELISA plate followed by incubation at 4 °C overnight. The following day, the wells were rinsed 3 times with IX PBS and excess liquid was discarded by flipping over the ELISA plates. The wells were blocked with 200 µL of 2 per cent BSA blocking solution for 2 hours at 37 °C. The wells were washed thrice with 1X PBS and excess buffer was discarded by flipping over the ELISA plate. $1x10^{12}$ PEG-NaCl precipitated phages from the end of each round of biopanning was prepared in 2 per cent BSA solution and 200 µLof this was added per well. The ELISA plate was incubated for 90 minutes at room temperature. The solution was discarded and the wells were washed 3 times with 1X PBS-0.05% Tween-20 followed by 3 times with 1X PBS. Anti M13 HRP conjugate was diluted in 2 per cent BSA and 200 µL of this was added per well. The ELISA plate was incubated for 90 minutes at room temperature. The anti M13 HRP conjugate was discarded and washed 3 times with 1X PBS 0.05% Tween 20 followed by 3 times with 1X PBS. Substrate was added to each well (150 µL) and incubated in dark for 30 minutes followed by addition of 50 µL of 1N HCL each to stop the solution. Then the plate was read at 450nm, 60 minutes after addition of the substrate. Fig. 1, represents the overview of the ELISA workflow.

Generation of Monoclones and ELISA

Phages obtained after fourth round of biopanning were used to generate monoclonal antibodies by standard protocol (Chandrashekara, 2006). Forty individual colonies from fourth round selection plate were picked and phages carrying scFv's were isolated. The obtained phages from the individual colonies were

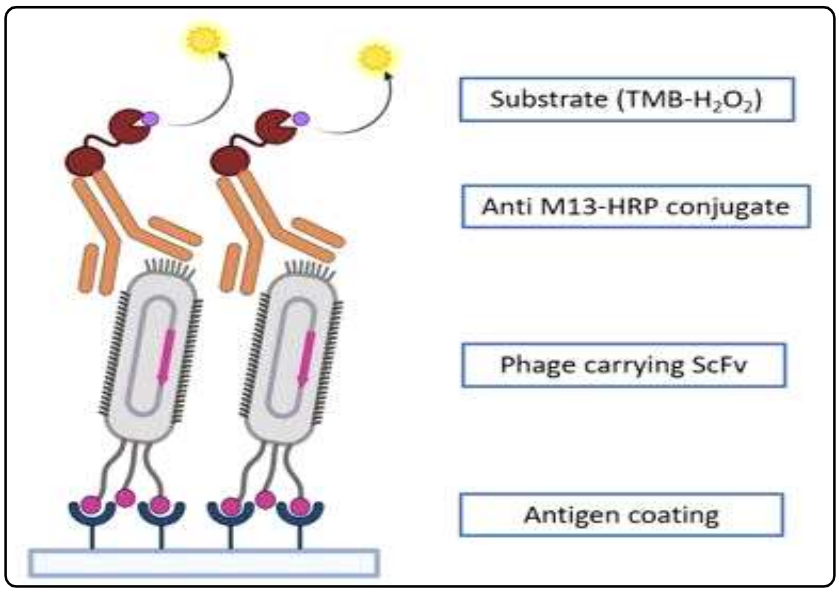

Fig. 1: Schematic representation of workflow of ELISA (Illustration was created using www.biorender.com)

assessed for their antigen binding affinity using ELISA which was carried out following the same procedure as outlined in the section on polyclonal ELISA. The phage displaying the highest absorbance value was selected for further analysis.

Screening by PCR Amplification of scFv Gene, Restriction Profiling and Characterization

The plasmid was isolated from selected colonies and PCR was performed to amplify scFv gene. The following primers were used *viz.,* forward primer LMB3- 5'CAGGAAACAGCTATGAC3' and reverse primer pHEN- 5'CTATGCGGCCCCATTCA3' for the amplification of scFv and PCR reaction was performed in a final volume of 20 µL. The mixture contains 10.0 µL of Takara PCR master mix (2x Premix), 1.0µL of each primer (10 pmol /µL), 1.0 µL of template DNA (conc. was around 50 ng/µL) and 7.0 µL nuclease-free water. The PCR condition was given initial denaturation at 95 °C for 10 mins and 35 cycles of 1 min denaturation at 94 °C, 1 min annealing at 57 °C and 2 min extension at 72 °C, followed by a final extension at 72 °C for 10 mins. The amplified PCR products were separated using 1.0 per cent agarose gel in 1X TAE buffer along with the 1kb ladder. Further the amplified product from the selected sample was sent for Sanger sequencing.

Results and Discussion

Antigen Preparation

The bacterial expression system is one of the most widely preferred method for producing the target protein intended for use as immunogens in antibody generation (Gulati- Sakhuja *et al.,* 2009). Heterologous expression coupled with affinity chromatography enables large scale production of desired protein with high degree of purity.

In present study, the AYWB SAP11 protein was utilized as antigen and mass production was achieved using bacterial expression system. The expressed and purified protein was analyzed on SDS-PAGE. The presence of distinct band ~16 kDa in all the four eluted samples, indicates successful expression and presence of single band represents high purity of eluted protein (Lane 4-7, Plate 1). The second and third eluted fractions showed higher band intensity (Lane 5&6, Plate 1). These samples were then

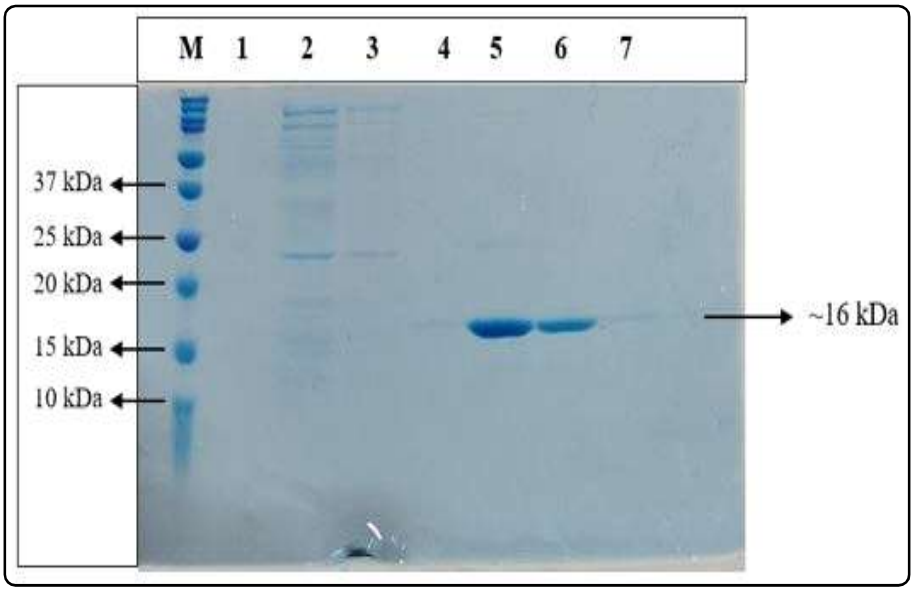

Plate 1 : SDS-PAGE analysis of purified SAP11 protein

(Lane M: 10-250kDa marker; Lane 2: Flow through collected during the Ni-NTA column purification; Lane 3: Washed protein eluent collected during the Ni-NTA column purification; Lane 4: First elution of protein; Lane 5: Second elution of protein; Lane 6: Third elution of protein; Lane 7: Fourth elution of protein)

dialysed in 1X PBS. The final concentration of protein was 0.5 mg/mL as quantified by Bradford method and this preparation was used inphage display.

Previous studies have that proteins expressed in bacterial system have been effectively utilized for development of serological based assay to detect phytoplasma diseases. Shahryari *et al.* (2010) successfully obtained higher expression of IMP of *Candidatus* Phytoplasma auusing *E. coli* BL21 (DE3) cells and developed both double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and dot immunosorbent assay (DIBA) to detect witches' broom disease of lime. Flippin *et al.* (2019) employed *E. coli* expression system for largescale production of flavescence dorée IMP protein and developed polyclonal antibodies for diagnosing the grapevine phytoplasma disease.

Similarly, in majority of plant virus detection assay involving serological techniques, the primary source for obtaining high purity antigen is mainly achieved through bacterial heterologous expression system. The successful example includes the expression of *Sugarcane streak mosaic virus* coat protein (SCSMV-CP) in *Escherichia coli* BL21 (DE3) and Rosetta-gami (DE3) pLysS host (Hamdayanty *et al*., 2016), the expression of coat protein of *Sugarcane Mosaic Virus* (SCMV) in *Escherichia coli* BL21 (DE3) (Astuti *et al.,* 2019) and *Peanut stunt virus* coat protein (PSV-CP) expression in *E. coli* BL21 (DE3) (Nemat *et al*., 2020), allaimed at developing efficient polyclonal antibodies for immuno diagnostic applications.

Isolation, Validation and Phage Yield Estimation of M13 K07 Helper Phage and Tomlinson's Phage Library

Isolated M13 K07 helper phages are validated using the reinfection to the host *E. coli* K12 and observed for the formation of plaques on top of the bacterial lawn. The appearance of clear plaques on the *E. coli*, confirmed the activity of the isolated helper phages (Plate 2) and phage yields were estimated using plaque count and absorbance measurements (Table 1).

The plaque formation assay is a crucial method for confirming the virulence activity of the phages. In addition, this assay is helpful in phage differentiation, plaque purification, plaque morphological studies and enumeration (determination of pfu/mL) of phages (Glonti and Pirnay, 2022).

The active M13K07 phages were used as helper phages for isolation of Tomlinson's phage library. The isolated scFv displaying phages were serially diluted, re-infected to *E. coli* K12 host and plated on selective antibiotic to validate the proper isolation. The phage yields, estimated from absorption spectroscopic method are presented in Table 1.

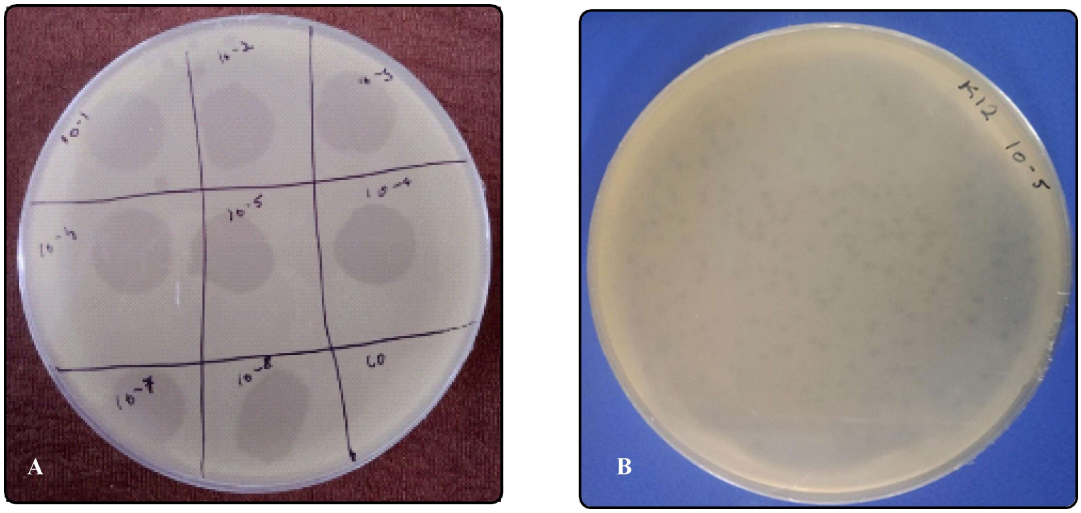

Plate 2 : Infectivity assay of M13 K07 helper phages

(A: Spot test method; B: Double agar layer method)

TABLE 1

Estimated phage yield of isolated helper phages and Tomlinson's phage library

Type of phage	Colony count method		Absorption spectroscopy method		
	Number of plaques	Phage yield	OD 269	OD 320	Phage yield
M13 K07	248 (@10^{-7})	2.48×10^{13}	11.907	0.470	1.072×10^{14}
Tomlinson's phage library	-	-	7.048	0.508	4.616×10^{13}

Selection of Phages Carrying Antibody Specific to SAP11 Protein

To select the antibodies specific to SAP11 protein from scFv library, the biopanning technique was performed. Four rounds of biopanning were conducted and resultant colonies obtained after each round of biopanning are shown in Plate 3. Subsequently, phages were isolated from the colonies obtained at the end of each biopanning round and used in the serological based assays.

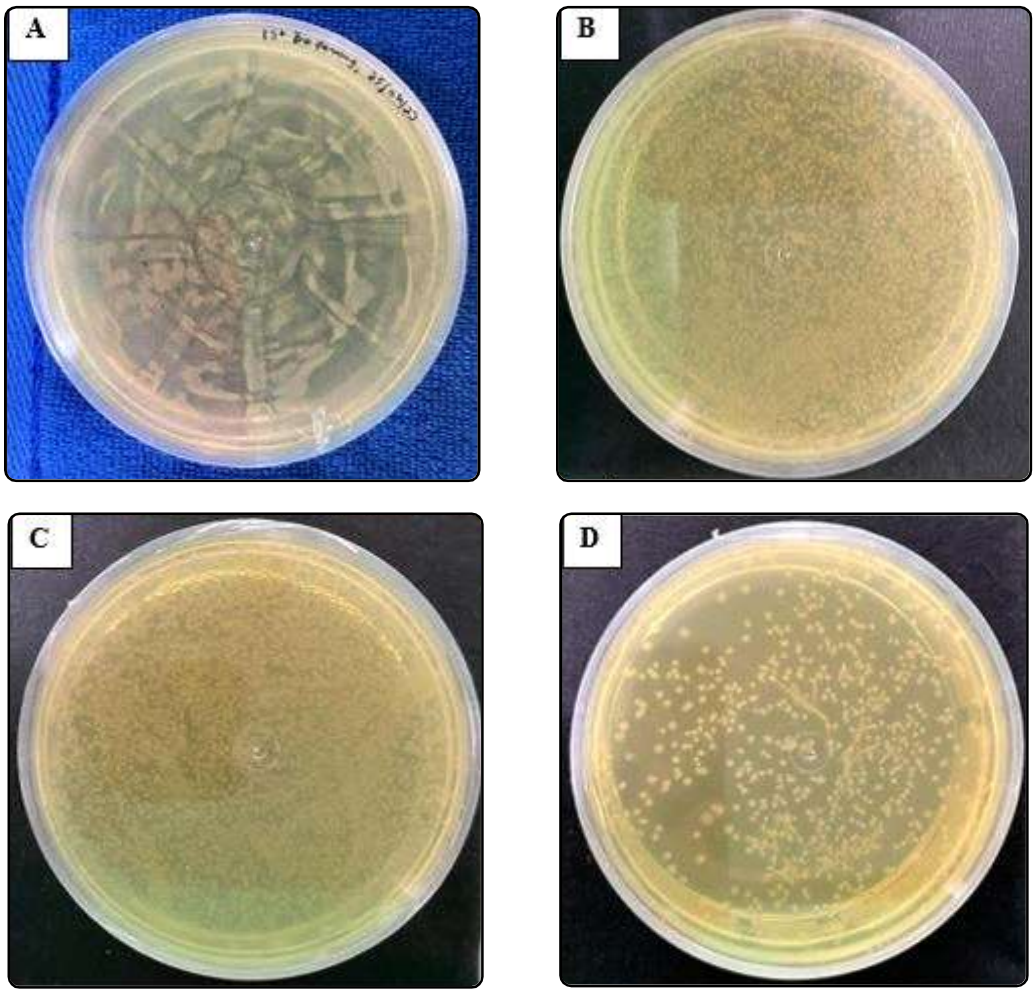

Plate 3 : Eluted phages were plated on selection media after each round of biopanning

(A: After first round of BP; B: After second round of BP; C: After third round of BP; D: After fourth round of BP)

Dot Blot Assay

A dot blot technique is simple and quick qualitative assay that can be employed to evaluate the effectiveness of the antibodies and the detection system. It can also be used to determine the appropriate starting concentration of primary antibody for ELISA and Western blot (Bio-techne protocol, 2015).

Initially a dot blot assay was performed to confirm the binding between phage-displayed scFv and the antigen. The SAP11 antigen was serially diluted starting from 1µg/mL to 0.125 µg/mL and 10µL of each dilution were dotted onto nitrocellulose membrane. The phages from both third and fourth rounds of BP showed visible blue coloration at the dotted spots across all dilutions (Plate 4) that indicates positive interaction.

Polyclonal ELISA

An ELISA was performed to evaluate which round of phages has highest affinity towards SAP11, this represents the polyclonal ELISA. The phages isolated in the fourth round showeds lightly higher absorbance compared to third round of BP at 2.5µg/mL of antigen coated (Fig. 2). Based on this result, the fourth round of phages were selected for subsequent monoclonal antibody generation.

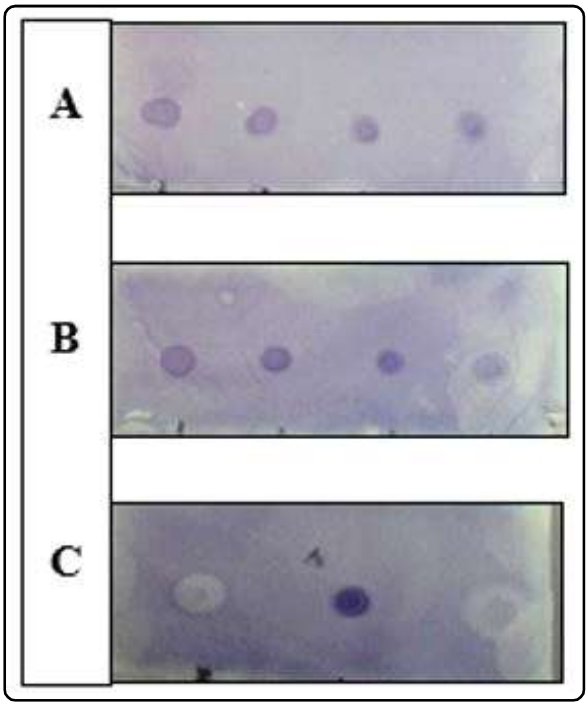

Plate 4 : Interaction of SAP11 antigen with phage derived antibody using dot blot assay

[A: SAP11 antigen was dotted and phage derived from third biopanning was used as primary antibody; B: SAP11 antigen was dotted and phage derived from fourth biopanning was used as primary antibody; C: Controls of the experiment (a) BSA (Negative control), (b) Phage derived antibody (3rd BP) (positive control) and (c) PBS (Negative control)]

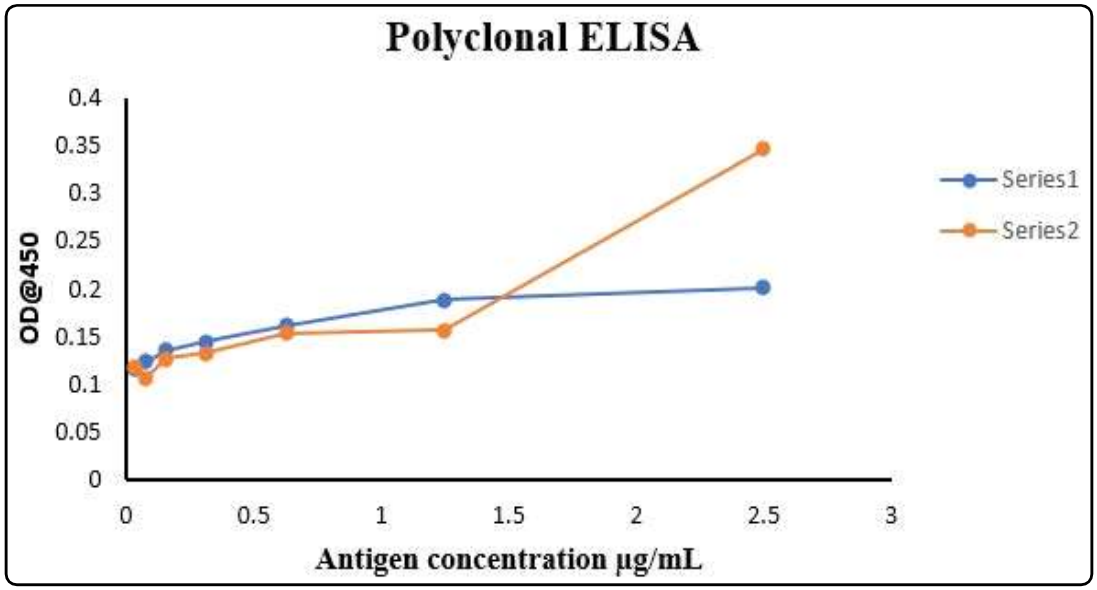

Fig. 2: Polyclonal ELISA
(Series 1 : Phages obtained from third biopanning; Series 2 : Phages obtained from fourth biopanning)

The research conducted by Rabinal *et al.* (2015) reported higher ELISA reading in fourth biopanning compared to the third round during development of polyclonal antibody against the Cry1Ac protein.

Monoclonal ELISA

In present research, 40-individual colony from the plate was cultured separately, amplified and the corresponding phages were isolated. The phage yield was estimated using spectrophotometry (Table 2) and ELISA was performed to select the phage displaying scFv that has highest affinity towards the SAP11. Among 40 colonies tested, colony 1 and colony 25 given highest OD value of 1.083 and 0.986, respectively (Table 2, Fig. 3). Further, colony 1 and 25 were plated on the selection media, followed by phagemid isolation and amplification using the primer pair of LMB3 and PHEN, targeting phage displayed scFv. However, the selected two colonies were failed to amplify the expected ~935bp amplicon; instead amplicon was observed at 329bp (Plate 5A). Additionally, restriction digestion with *Sfi* I and *Not* I enzyme failed to release the scFv insert suggesting the absence of desired insert (Plate 5B).

To confirm the result, a monoclonal ELISA was repeated with the 20 best colonies (those with OD value above 0.3 according to the previous monoclonal ELISA), which were freshly amplified and isolated. The ELISA was conducted for the selected 20 colonies using three different dilutions according to the phage yield: higher concentration of phages (adjusted to

Table 2
Phage yield, volume of phage preparation required and ELISA reading for first batch of monoclonal phages

Colony Number	Absorbance at 269nm	Absorbance at 320nm	Calculated Absorbance value	Calculated phage yield	Volume required for normalize to $1x10^{12}$	ELISA reading	
						Antigen used (Diluted) 0.625 µg/mL	Antigen used (Conc.) 1.25 µg/mL
1	0.024	0.001	0.024	1.67×10^{11}	5.97	0.444	1.083
2	0.020	-0.001	0.021	1.48×10^{11}	6.76	0.349	0.361
3	0.020	0.001	0.019	1.34×10^{11}	7.46	0.184	0.177
4	0.049	0.002	0.047	3.30×10^{11}	3.03	0.189	0.47
5	0.021	0.000	0.021	1.51×10^{11}	6.63	0.357	0.249
6	0.040	0.006	0.035	2.46×10^{11}	4.07	0.681	0.523
7	0.050	0.033	0.017	1.19×10^{11}	8.40	0.245	0.189
8	0.032	0.002	0.030	2.11×10^{11}	4.73	0.458	0.588
9	0.029	0.002	0.028	1.96×10^{11}	5.11	0.36	0.683
10	0.028	0.000	0.027	1.92×10^{11}	5.21	0.187	0.323
11	0.028	0.001	0.027	1.90×10^{11}	5.26	0.178	0.395
12	0.011	-0.001	0.012	8.32×10^{10}	12.02	0.145	0.142
13	0.041	0.030	0.011	7.93×10^{10}	12.60	0.173	0.172
14	0.008	-0.001	0.008	5.95×10^{10}	16.81	0.112	0.122
15	0.007	-0.001	0.008	5.60×10^{10}	17.86	0.181	0.118
16	0.028	0.001	0.027	1.91×10^{11}	5.24	0.461	0.636
17	0.003	-0.002	0.005	3.51×10^{10}	28.50	0.145	0.12
18	0.021	0.000	0.021	1.48×10^{11}	6.77	0.211	0.182
19	0.051	0.028	0.024	1.67×10^{11}	6.00	0.209	0.307

Continued....

TABLE 2 Continued....

Colony Number	Absorbance at 269nm	Absorbance at 320nm	Calculated Absorbance value	Calculated phage yield	Volume required for normalize to $1x10^{12}$	ELISA reading	
						Antigen used (Diluted) 0.625 µg/mL	Antigen used (Conc.) 1.25 µg/mL
20	0.019	0.018	0.002	1.19×10^{10}	83.83	0.131	0.148
21	0.048	0.002	0.046	3.26×10^{11}	3.06	0.727	0.712
22	0.012	-0.017	0.029	2.04×10^{11}	4.89	0.369	0.229
23	0.017	0.001	0.015	1.09×10^{11}	9.15	0.224	0.142
24	-0.001	-0.018	0.017	1.20×10^{11}	8.30	0.321	0.118
25	0.036	0.002	0.035	2.44×10^{11}	4.10	0.959	0.986
26	0.019	-0.015	0.035	2.44×10^{11}	4.10	0.404	0.22
27	0.009	-0.016	0.025	1.79×10^{11}	5.57	0.347	0.209
28	0.038	0.003	0.035	2.45×10^{11}	4.09	0.521	0.574
29	0.041	0.004	0.037	2.60×10^{11}	3.85	0.764	0.496
30	0.042	0.004	0.039	2.74×10^{11}	3.65	0.63	0.424
31	0.017	-0.015	0.032	2.23×10^{11}	4.49	0.219	0.128
32	0.001	-0.017	0.018	1.24×10^{11}	8.08	0.3	0.147
33	0.017	0.013	0.004	3.12×10^{11}	32.05	0.247	0.152
34	0.026	-0.011	0.038	2.67×10^{11}	3.74	0.221	0.287
35	0.066	0.018	0.048	3.41×10^{11}	2.93	0.49	0.417
36	0.012	-0.017	0.029	2.01×10^{11}	4.97	0.187	0.301
37	0.028	0.013	0.015	1.07×10^{11}	9.34	0.218	0.165
38	0.038	0.012	0.026	1.84E + 11	5.44	0.318	0.282
39	0.030	0.017	0.013	9.26E + 10	10.80	0.302	0.135
40	0.030	0.002	0.029	2.01E + 11	4.97	0.734	0.382

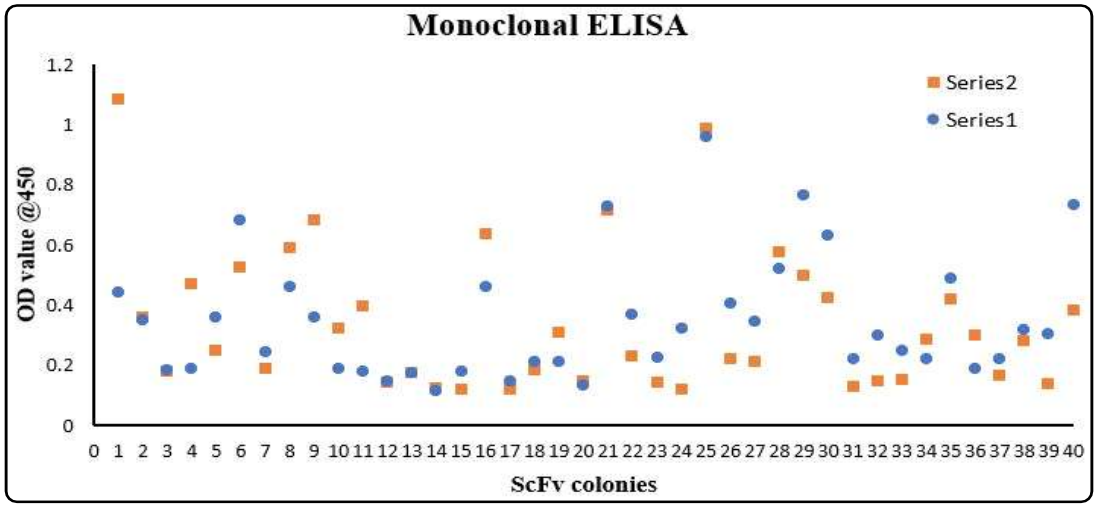

Fig. 3 : Monoclonal ELISA of first monoclonal batch

(Series 1: 0.625 ġg/mL of antigen (SAP11) concentration; Series 2: 1.25 ġg/mL of antigen (SAP11) concentration)

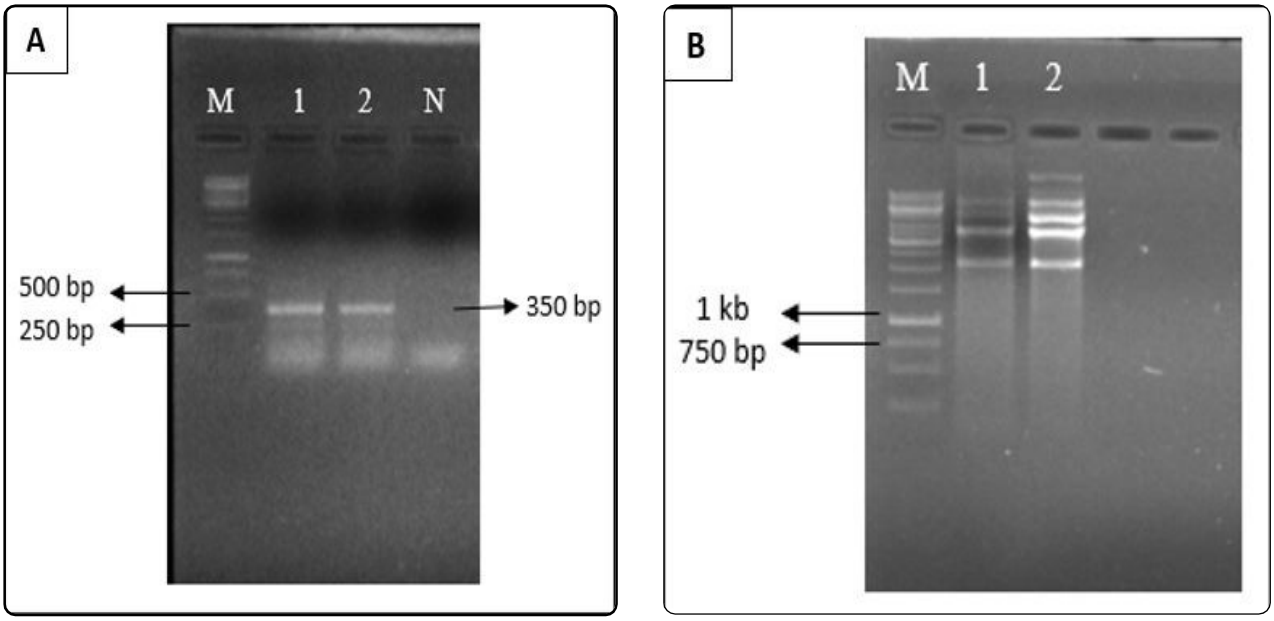

Plate 5 : Analizing the presence of ScFv gene in selected phagemid

[A) PCR amplification using LMB3 and pHEN primer pair (Lane M: 1kb Ladder, Lane 1: Phagemid from colony 1, Lane 2: Phagemid from colony 25); B) Restriction digestion of phagemid using Sfi1 and Not 1 enzymes (Lane M: 1kb Ladder, Lane 1: Phagemid from colony 1, Lane 2: Phagemid from colony 25)]

$5\times10^{\wedge11}$ phage/mLas per the spectrophotometer reading), a lower concentration of phages ($2.5\times10^{\wedge11}$ phage/mL) and a fixed volume (12 µL of phage preparation). Table 3 summarizes the OD value of 20 colonies, corresponding volumes of phage preparation used in each dilution for the ELISA assay and absorbance value obtained in ELISA. Fig. 4, represents result of ELISA absorbance value

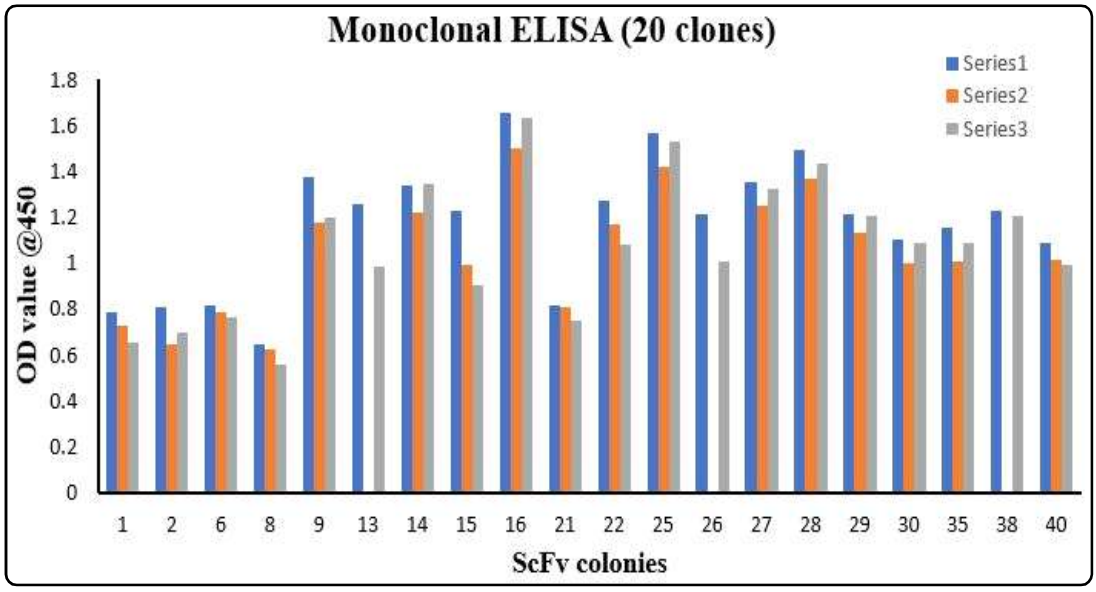

Fig. 4 : ELISA reading of second monoclonal batch with different dilutions of primary antibody (phages)

[Series 1: Higher concentration of phages (adjusted to $5\times10^{\wedge11}$ phage/mL); Series 2: A lower concentration of phages ($2.5\times10^{\wedge11}$ phage/mL); Series 3: Fixed volume of phages (12 µL of phage preparation)]

TABLE 3

Phage yield, volume of phage preparation required and ELISA reading for second batch of monoclonal phages

Colony No.	Absorbance at 269nm	Absorbance at 320nm	Calculated Absorbance value	Phage yield	Volume required for normalizing to 0.5x10^12	ELISA reading			
						Antibody used at conc. of 0.5x10^12 number of phage	Antibody used at conc. of 0.25x10^12 number of phage	Antibody used at fixed volume (12 µL) of phage	Antibody used at fixed volume (5 µL) of phage
1	3.508	0.474	3.034	2.14 x10 13	23.35	0.786	0.7285	0.6535	
2	5.142	2.236	2.906	2.05 x10 13	24.37	0.8045	0.6465	0.6975	
6	32.475	-0.411	2.886	2.04 x10 13	24.54	0.8115	0.788	0.765	
8	3.381	1.416	1.965	1.39 x10 13	36.05	0.6465	0.6265	0.5595	
9	3.944	1.649	2.295	1.62 x10 13	30.86	1.373	1.1745	1.195	0.408
13	5.168	3.442	1.726	1.22 x10 13	41.04	1.2605	0	0.988	
14	5.287	1.798	3.489	2.46 x10 13	20.30	1.3395	1.2205	1.3445	0.51
15	3.167	2.056	1.111	7.84 x10 12	63.76	1.225	0.989	0.9065	
16	4.795	0.86	3.935	2.78 x10 13	18.00	1.6525	1.4985	1.6345	1.248
21	2.78	0.611	2.169	1.53 x10 13	32.66	0.8165	0.8055	0.752	
22	3.105	1.025	2.08	1.47 x10 13	34.05	1.274	1.1715	1.0795	
25	3.831	0.349	3.482	2.46 x10 13	20.34	1.567	1.4215	1.5285	0.744
26	5.199	3.225	1.974	1.39 x10 13	35.88	1.215	0	1.006	
27	3.998	1.267	2.731	1.93 x10 13	25.94	1.3545	1.247	1.3225	0.442
28	4.208	1.847	2.361	1.67 x10 13	30.00	1.495	1.3705	1.4345	0.858
29	2.875	0.812	2.063	1.46 x10 13	34.34	1.2125	1.1305	1.203	0.934
30	3.527	1.035	2.492	1.76 x10 13	28.42	1.1	0.9965	1.088	
35	2.969	0.403	2.566	1.81 x 10 13		27.60	1.153	1.007	1.088
38	4.796	3.141	1.655	1.17 x10 13	42.80	1.2275	0	1.208	0.24
40	1.117	-0.787	1.904	1.34 x10 13	37.20	1.089	1.0165	0.9905	

of three dilutions of phages. Based on the ELISA absorbance, 8 colonies were shortlisted and ELISA was repeated in order to confirm their reproducibility. This time, the experiment was done with 5 µL volume of phage preparation. Among 8 colonies assessed, the colony number 16,25,28 and 29 showed higher absorbance value compared to the others (Table 3, Fig. 5). The phagemids from these four colonies were

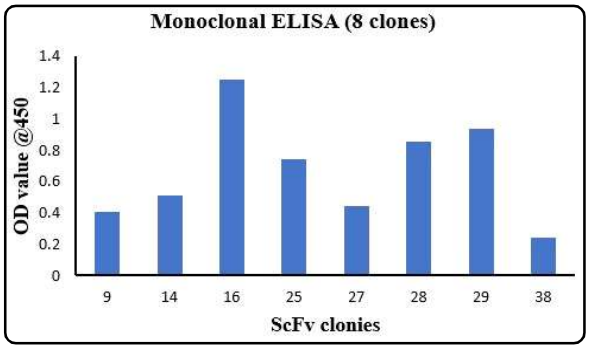

Fig. 5 : ELISA reading of selected phage colonies from second monoclonal batch

isolated and PCR was performed using the LMB3 and PHEN primers. Unfortunately, the result ended up with the amplicon size of 329bp, indicating that the phagemids did not encode the scFv (Plate 6).

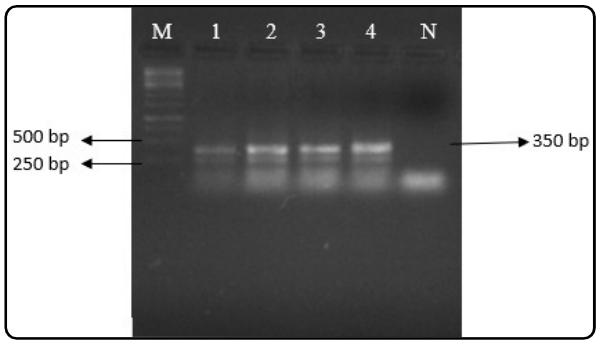

Plate 6 : PCR amplification of phagemids from second monoclonal batch

(Lane M1: 1kb ladder; Lane 1: Phagemid from colony 16; Lane 2: Phagemid from colony 25; Lane 3: Phagemid from colony 28; Lane 4: Phagemid from colony 29; Lane N: negative control)

As a next part of trouble shooting, the entire process starting from antigen preparation to performing the four rounds of biopanning process was repeated. A small change in procedure was made. The obtained colonies after the fourth round of biopanning were subjected to colony PCR first rather than being analyzed using EILSA. A total of 288 colonies were screened using PCR, out of which only 16 colonies amplified the expected band (~935bp), whereas 12

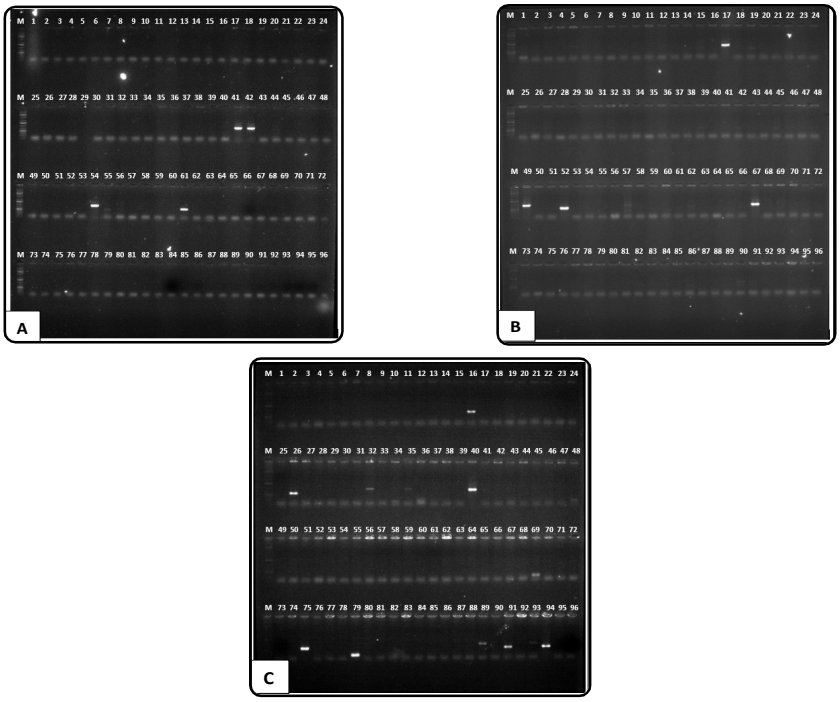

Plate 7 : PCR amplification phage colonies with LMB3 and pHEN

[A) Phage colonies 1-96; B) Phage colonies 97-192 and C) Phage colonies 193-288]

colonies amplified band of a size less than 700bp and empty phagmids amplified at 329bp (Plate 7). The percentage of confirmed colonies was only 5 per cent, suggesting that either the scFv present in the library had low affinity towards the SAP11 antigen or that the biopanning process was ineffective. Further, the phagemids were isolated from these 16 colonies and the PCR was performed. Out of which, the amplification was observed for 15 colonies at the expected size (Plate 8) and subsequently ELISA was carried out. Initially, colonies 1 and 14 showed higher OD values compared to the others; however, the results were not consistent when the ELISA was repeated. Moreover, restriction digestion did not release the scFv insert and sequencing did not yield reliable reads.

Phage display technique has been applied in plants mainly to detect the diseases using serological based assays. Few examples include, Yuan *et al.* (2016) screened scFv library using monoclonal ELISA and developed scFv antibodies against the surface proteins of *Ca. Liberibacter asiaticus* to detect Huanglongbing disease of citrus using phage display technique. Raesi *et al.* (2020) used monoclonal ELISA for testing individual clones and developed scFv against the *chickpea chlorotic dwarf virus* using Tomlinsons library. Shahmirzaie *et al.* (2020) evaluated 95 clones using monoclonal ELISA in which 10 scFv clones showed strong affinity against the nucleocapsid protein and developed efficient diagnostic platform to detect *fig mosaic virus*.

Whereas, in the present study, due to series of inconsistent ELISA results, absence of proper amplification in PCR and failure to release the scFv insert upon digestion, authors found it challenging to proceed further to complete the monoclonal development for the SAP11 antigen using the phage display approach. The work progressed up to the development of polyclonal antibodies as reliable results were obtained only in the polyclonal ELISA, hence the isolated phages from fourth round of biopanning may be further utilized for validation with field sample.

Several researches found difficulties during the biopanning process. In one of scientific platform, Yuehua (2018) stated that during the first round of biopanning, 80 per cent of the colonies found to be empty upon colony PCR analysis, however in further rounds of selection, a higher proportion of positive clones were obtained. Whereas, in our study, even after fourth round we observed only 5 per cent of colonies carries the phagemid which was very low to continue the process of mAB development.

The authors conclude that the difficulties encountered during the development of monoclonal antibodies may be due to inefficiency in biopanning process, lack of affinity between the target antigenand the scFv library used in the current research, or nonspecific binding of antigen to other regions of the phage particle. The present investigation is the first report where an attempt has been made to develop monoclonal antibodies against the phytoplasma protein using phage display approach. Here, we present the outcome of the large-scale production of high purity antigen, development of polyclonal antibodies using phage display and results of unsuccessful attempts in the monoclonal development, along with few trouble shooting insights, that may prove valuable for the future researchers to work on these aspects.

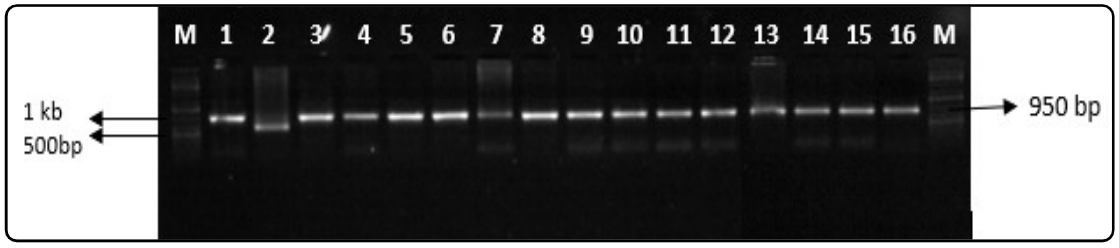

Plate 8 : PCR amplification of phagemids of selected sixteen colonies

(Lane M: 1kb ladder; Lane 1-16; Phagemid from 16 clones)

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Standardization of Media for *In vitro* Culture Establishment and Shoot Multiplication of the Endangered Orchid *Eulophia nuda* Lindl.

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Abstract

A systematic investigation was undertaken to optimize *in vitro* propagation protocols for *Eulophia nuda* Lindl. an endangered terrestrial orchid of tropical Asia notable for its medicinal properties. *In vitro* culture techniques provide an effective approach for the propagation and conservation of endangered species, while the optimization of culture media is essential for successful seed development. Six solid media formulations: Murashige and Skoog (MS) full and half strength, Lindemann full and half strength and Orchimax full and half strength were evaluated for seed germination and development of protocorm-like bodies (PLBs). Orchimax full strength media supported the highest seed germination rate (92.20 %) and the most rapid germination occurring at 66.60 days after culturing (DAC). PLBs were established earliest (66.60 DAC) and most profusely (91.12 %) on the same media. Optimal shoot induction was achieved using Orchimax full strength media supplemented with BAP (1.0 mg/L) and NAA (0.5 mg/L), which resulted in early shoot initiation (11.20 days), maximum shoot number (4.60 per explant) and maximum shoot length (4.30/ cm) at 90 DAC. These findings establish an efficient *in vitro* propagation system, facilitating large-scale multiplication and conservation of this threatened orchid species.

Keywords : Conservation, Culture media, *In vitro*, Protocorm-like bodies, Seed germination

The Orchidaceae family represents one of the largest and most diverse families of angiosperms, encompassing about 28,000 species that are globally distributed across various ecosystems with complex ecological interactions (Fonseka, 2020). Terrestrial orchids suchas *Eulophia nuda* Lindl. are significant from both environmental and ethnomedicinal perspectives, particularly in biodiversity hotspots like the Western Ghats and Northeast India (Hada *et al*., 2020). The tubers of *E. nuda* have been traditionally used for medicinal purposes, including the treatment of tumours, blood diseases, scrofulous neck glands and respiratory ailments. These traditional uses highlight their potential pharmacological benefits (Misra *et al*., 2023).

Despite its economic and ecological value, the population of *E. nuda* have declined due to habitat degradation, climate change and overharvesting driven by high demand for medicinal use (Ticktin *et al*., 2023). Moreover, orchid seed germination in natural habitat is limited by the species'minute dust-like seeds that lack endosperm. Germination depends on specific mycorrhizal fungi that support nutrient provision during early developmental stages (Zhao *et al*., 2021). This obligate mutualism and seed

physiology contribute to low natural regeneration and recruitment in wild populations (Fonseka, 2020).

Due to these constraints, asymbiotic seed culture has emerged as a vital tool capable of circumventing mycorrhizal dependence by providing sterile, nutrient enriched environments conducive to seedling development (Bhowmik and Rahman, 2020a). While Murashige and Skoog (MS) media and Lindemann media are widely used, their effectiveness varies among orchid species, particularly terrestrial orchids such as *E. nuda* (Kaladharan *et al*., 2024). Recently, proprietary media like Orchimax, enriched with organic nitrogen sources such as tryptone and buffering agents like activated charcoal, have demonstrated enhanced seed germination and protocorm-like bodies (PLBs) development across diverse terrestrial orchids (Yahya *et al*., 2024).

Regardless of broader applications in orchid tissue culture, few studies have rigorously optimized culture media specifically for *E. nuda*, a species categorized as endangered (Dawande and Gurav, 2015). This work systematically compares MS media, Lindemann media and Orchimax media, both at full and half strength, to establish a reproducible and efficient propagation protocol capable of supporting the conservation and sustainable use of this species. This research supports ongoing efforts within global botanical conservation frame works that emphasize biotechnological interventions, including micropropagation and *ex situ* germplasm preservation, for endangered orchid species (Jolman *et al*., 2022 and Kaladharan *et al*., 2024).

Material and Methods

Plant Material Collection

Mature specimens of *E. nuda* were collected from the Halasi village forest area (15°31’50"N, 74°34’24"E) in Belagavi, Karnataka, India and their identity was confirmed by Dr. A.N. Shringeshwar, Botanist at the Mahatma Gandhi Botanical Garden, University of Agricultural Sciences, GKVK, Bengaluru.

In vitro Seed Sterilization and Culture Initiation

The collected specimens (Plate 1) were subsequently maintained in the orchidarium at the Plant Tissue Culture Laboratory, Department of Horticulture, UAS, GKVK, Bengaluru. Following hand pollination, seed capsules were harvested 150 to 180 days after pollination to optimize embryo maturity. The immature capsules were initially placed in a sterile bottle containing 100 to 200 ml of distilled water with 1 to 2 drops of Tween 20 and sterilized for 30 minutes

Plate 1 : Morphological features of *E. nuda*: (A) Whole plant with tuber and roots; (B) Flower

with intermittent shaking. They were washed three times with sterile distilled water for five minutes each to remove all traces of Tween 20 from the surface of the pods. Later, the capsules were treated with 0.2 per cent Bavistin solution, shaken continuously for 20 minutes and rinsed three times with sterile distilled water for 5 minutes each. After, the capsules were transferred to a sterile bottle containing freshly prepared 0.1 per cent mercuric chloride solution, where they were soaked for three minutes with intermittent shaking. The solution was drained and the explants were washed five times with sterile distilled water to remove any remaining chemical residues. Excess moisture was carefully removed using sterile filter paper placed on sterile petri dishes. In the end, the capsules were dipped in 70 per cent ethanol and flamed for five seconds.

Seed Germination and Development of PLBs on Various Culture Media

Six media treatments were prepared (Table 1): Murashige and Skoog (full and half strength), Lindemann (full and half) and Orchimax (full and half strength) media and they were supplemented with 6 g/L agar and pH was adjusted to 5.70. Sucrose was added at 30 g/L (Budensha *et al*., 2025) in Murashige and Skoog media (MS) and 20 g/L in Lindemann and Orchimax media. Orchimax media was enriched with tryptone, MES buffer and activated charcoal (Yahya *et al*., 2024). The compositions and concentrations of all media are provided in Annexure (I to III). Ten replications per treatment were conducted with approximately 150 to 200 seeds (equivalent to half a spatula) transferred into each culture bottle using a sterile spatula under a laminar airflow chamber (LAF) (Bharath and Raju, 2023). Key parameters in the study included days to seed germination, days taken for PLBs formation, percentage seed germination and establishment of PLBs. Specifically, days to seed germination were recorded as the time from culture initiation to embryo emergence, while days to PLBs formation were noted from culture initiation until the appearance of compact, globular, creamywhite to pale green structures with distinct spherical protuberances. The percentage of germination was calculated 90 DAC as the number of seeds that formed embryos over the total seeds sown and the establishment of PLBs was evaluated at 120 DAC, representing the proportion of seeds producing PLBs out of the total seeds germinated. These parameters collectively provided insights into the temporal progression and efficiency of *in vitro* orchid seed germination and PLBs development.

Table 1

Culture media treatments for *in vitro* seed germination and PLBs development in *E. nuda*

Treatment	Solid media	Media strength
T_1	Murashige & Skoog (MS)	Full strength
T_2		Half strength
T_3	Lindemann orchid	Full strength
T_4		Half strength
T_5	Orchimax	Full strength
T_6		Half strength

Culture Condition

The cultures were maintained under cool white fluorescent illumination at a light intensity of 30-40 µmol m^{-2} s^{-1} (approximately 2000 lux) with a 16-hour photo period, at a constant temperature of 24 ± 2 °C.

Shoot Induction and Proliferation from PLBs using Different Plant Growth Regulators (PGRs)

Among all the tested media, Orchimax full strength media proved to be the most effective and was therefore selected for further experiments. PLBs of *E. nuda* measuring 4 to 5 mm, obtained from the previous experiment were used as explants. The influence of different concentrations of 6-Benzyl amino purine (BAP), Kinetin (KIN) and Naphthalene Acetic Acid (NAA), both individually and in combinations, on the growth and proliferation of PLBs was evaluated (Table 2). Observations were recorded for days taken for shoot initiation, number of shoots formed and shoot length (cm) after 90 DAC in the shoot induction media for *E. nuda*.

TABLE 2

Plant growth regulator treatments employed for shoot induction and regeneration from PLBs of *E. nuda*

Treatment	Concentration (mg/L)	
T_1	Control	
T_2	BAP	0.5
T_3		1.0
T_4		1.5
T_5	NAA	0.5
T_6		1.0
T_7		1.5
T_8	KIN	0.5
T_9		1.0
T_{10}		1.5
T_{11}	BAP+NAA	0.5 + 0.5
T_{12}		0.5 + 1.0
T_{13}		0.5 + 1.5
T_{14}		1.0 + 0.5
T_{15}		1.0 + 1.0
T_{16}		1.0 + 1.5
T_{17}		1.5 + 0.5
T_{18}		1.5 + 1.0
T_{19}		1.5 + 1.5
T_{20}	KIN+NAA	0.5 + 0.5
T_{21}		0.5 + 1.0
T_{22}		0.5 + 1.5
T_{23}		1.0 + 0.5
T_{24}		1.0 + 1.0
T_{25}		1.0 + 1.5
T_{26}		1.5 + 0.5
T_{27}		1.5 + 1.0
T_{28}		1.5 + 1.5

BAP- Benzylaminopurine, KIN- Kinetin,
NAA- Naphthalene Acetic Acid

Statistical Analysis

Statistical analysis was carried out using the F-test at a 1 per cent level of significance and the critical difference (CD) values were determined using the Online Statistical Analysis Tool (OPSTAT) software package.

RESULTS AND DISCUSSION

Effect of Different Basal Media on Seed Germination and PLBs Development

Days taken for Seed Germination : The composition and ionic strength of the culture media significantly influenced the time required for seed germination in *E. nuda* (Table 3). Early seed germination occurred on Orchimax full strength solid media (T_5) at 66.60 DAC, followed by Orchimax half strength solid media (T_6) at 70.90 DAC. In contrast, delayed germination was recorded on full strength Lindemann solid media (T_3) at 88.90 DAC.

TABLE 3

Effect of different culture media on days taken for seed germination in *E. nuda*

Treatments	Days taken for seed germination
T_1 - MS full strength solid media	79.60
T_2 - MS half strength solid media	74.10
T_3 - Lindeman full strength solid media	88.90
T_4 - Lindeman half strength solid media	83.90
T_5 - Orchimax full strength solid media	66.60
T_6 - Orchimax half strength solid media	70.90
F-Test	**
S. Em.±	0.80
CD at 1%	2.98

** Significant at P= 0.01

Germination Percentage (%) : The germination percentage of *E.nuda* seeds at 90 DAC demonstrated significant variation across the culture media tested. The highest germination rate of 92.20 per cent was observed on Orchimax full strength solid media, followed by 88.66 per cent on Orchimax half strength solid media. The lowest germination rate of 70.94 per cent, occurred on Lindemann full strength solid media (Fig. 1).

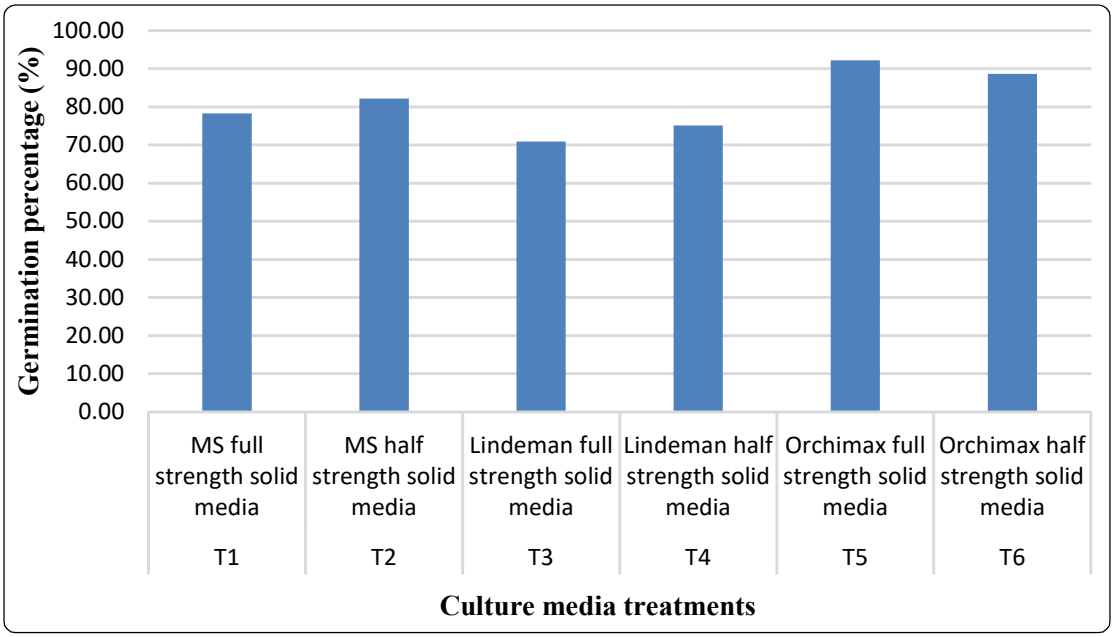

Fig. 1 : Influence of different culture media on germination percentage (%) in *E. nuda*

The exceptional effectiveness of Orchimax full strength solid media in promoting orchid seed germination, compared to other media, may be attributed to its carefully formulated composition tailored specifically for orchids. It provides an optimal balance of mineral salts, carbohydrates and organic additives such as tryptone and activated charcoal. Research has consistently demonstrated that orchid seed germination improves significantly in media that combine both organic and inorganic nitrogen sources (Stewart and Kane, 2016). In Orchimax media, tryptone acts as an organic nitrogen source, enhancing germination more effectively than other media. Furthermore, tryptone supplies readily available amino acids, while activated charcoal adsorbs phenolic compounds that might otherwise inhibit growth, thereby creating an ideal environment for early seed germination and development (Utami and Hariyanto, 2020).

Yahya *et al*. (2024) reported that *Phalaenopsis* spp. cultured on Orchimax media exhibited the earliest seed germination and the highest germination percentage among all media tested. Bektas and Sokmen (2016) similarly found enhanced germination of *Serapias vomeracea* on Orchimax media. Arcidiacono *et al.* (2021) compared seed germination of *Anacamptis longicornu* on Orchimax and MS media, observing a 95.50 per cent germination rate on Orchimax versus 21.40 per cent on MS media. In the same experiments with *Ophrys panormitana*, germination occurred exclusively on Orchimax media and failed on MS media. These differences were primarily attributed to variations in nutrient composition, particularly the form and concentration of nitrogen.

In *Ophrys reinholdii* subsp. *straussii,* Ahmadzadeh *et al*. (2025) reported highest average germination on Gamborg's B_5 quarter strength media (87.31%) and Malmgren media (83.83%), whereas MS quarter strength media (65.86%) and Orchimax media (60.50%) yielded significantly lower rates of germination. Under the same experiment, *Comperia comperiana* displayed uniformly low germination, peaking at 14.04 per cent on P723 media and dropping to 4.50 per cent on B_5 quarter strength media, likely reflecting species-specific nutritional requirements or physiological dormancy. These findings demonstrate that optimal asymbiotic seed germination media differ markedly among terrestrial orchid species, driven by genetic variation and seed composition. Following

germination, the developmental progression towards PLBs formation was strongly influenced by the same media composition.

Days Taken for PLBs Formation : In *E. nuda*, PLBs formation occurred earliest in Orchimax full strength solid media (80.10 DAC) followed by Orchimax half strength solid media at 84.10 DAC. In contrast, Lindemann full strength solid media showed the slowest formation at 97.40 DAC (Table 4).

TABLE 4

Effect of different culture media on days taken for PLBs formationfrom seeds of *E. nuda*

Treatments	Days taken for PLBs formation
T_1- MS full strength solid media	91.10
T_2- MS half strength solid media	87.40
T_3- Lindeman full strength solid media	97.40
T_4- Lindeman half strength solid media	94.40
T_5- Orchimax full strength solid media	80.10
T_6- Orchimax half strength solid media	84.10
F-Test	**
S. Em.±	0.71
CD at 1%	2.70

** Significant at P= 0.01

Establishment of Protocorm-like Bodies (%) : The establishment and early morphogenetic responses of *E. nuda* were significantly affected by the composition of the culture media, as demonstrated (Fig. 2 & Plate 2) by statistically significant differences in the percentage establishment of PLBs at 120 DAC. The highest establishment of PLBs was recorded in Orchimax full strength media (91.12%), followed by Orchimax half strength media (87.95%). Poor establishment of PLBs was noted in Lindeman full strength solid media (71.70%). In comparison, Lindemann media often resulted in slower PLBs formation and lower induction frequencies, likely due to a less optimal nutrient balance and absence of necessary organic additives. MS media are commonly used but may produce suboptimal results for orchids due to higher salt concentrations and lower organic compound content. This can delay early developmental stages, including the initiation of PLBs (Nowakowska *et al*., 2022). Raj *et al.* (2021) observed that PLBs induction occurred at 20 DAC on MS half strength media, whereas, PLBs induction was delayed until 66.60 DAC on Lindemann full strength media. This result underscores the critical role of media composition and strength in modulating PLBs formation timing in orchid tissue culture. PLBs formation and

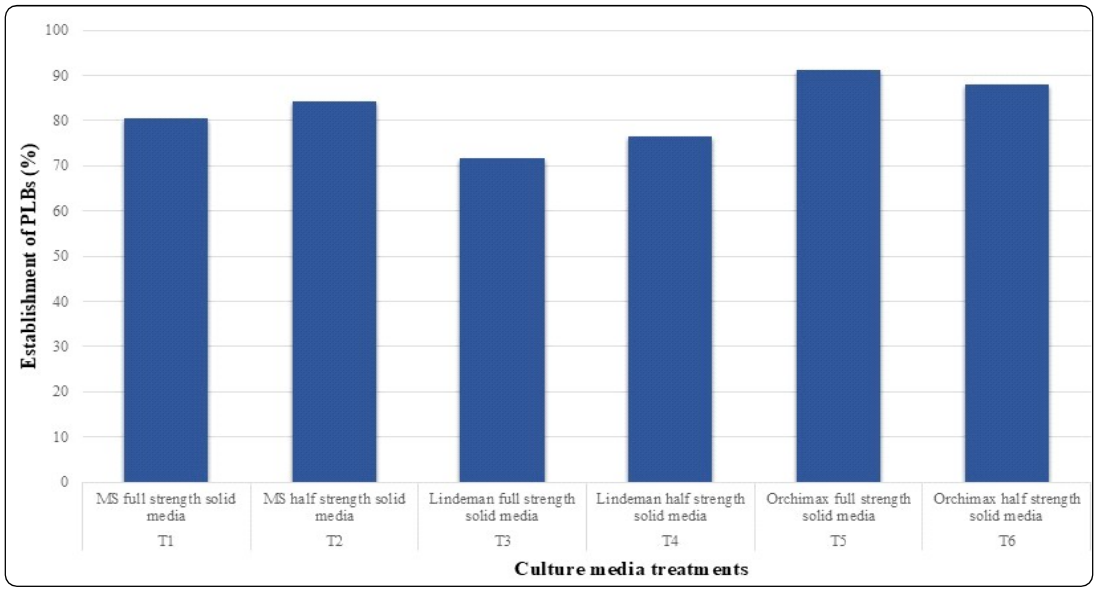

Fig. 2 : Influence of different culture media on the establishment of PLBs (%) from seeds of *E. nuda*

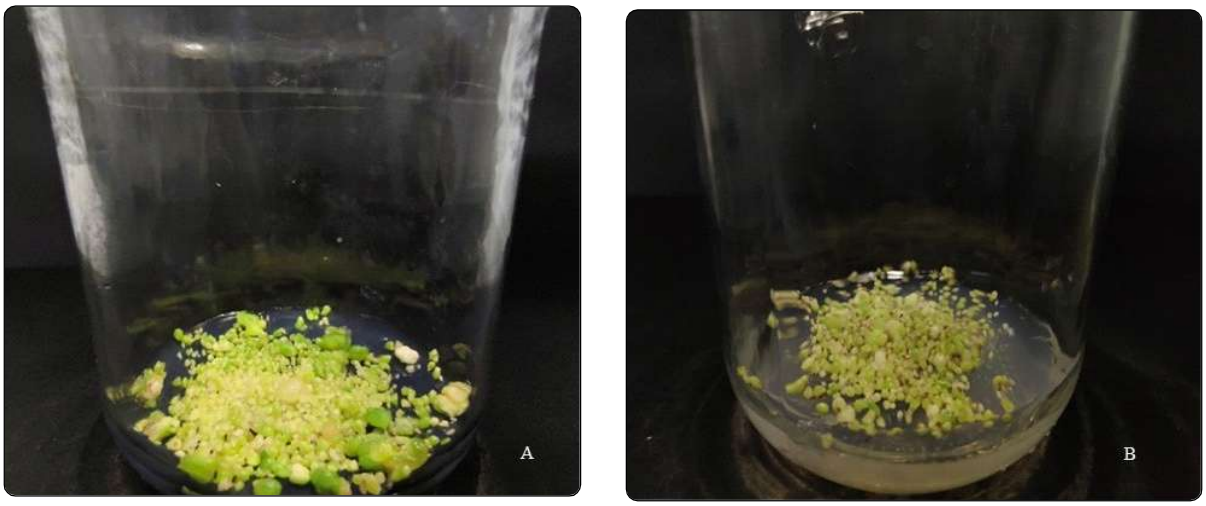

Plate 2 : Development of PLBs from seeds of *E. nuda*.

(A) In Orchimax full strength solid media and (B) Lindemann full strength solid media

establishment varied considerably among orchid taxa, reflecting species-specific responses to media composition. Optimizing nutrient and organic components to match each species' physiological and genetic characteristics is therefore crucial for maximizing propagation efficiency.

Once PLBs were established, their morphogenic potential was further assessed through shoot

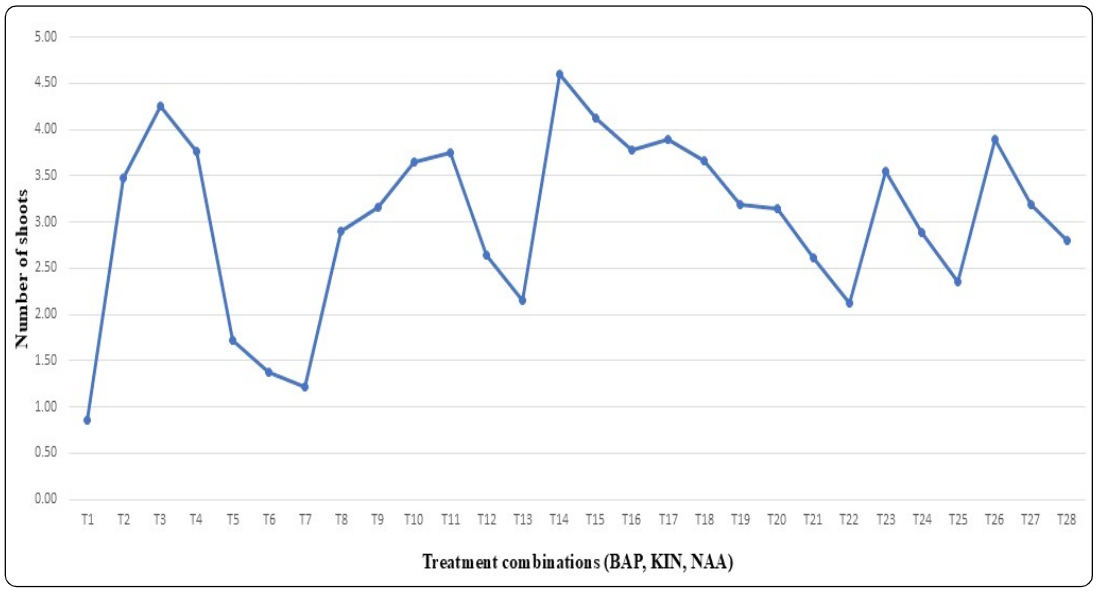

Fig. 3 : Influence of PGRs and their combinations on number of shoots per PLBin *E. nuda* at 90 DAC in shooting media

T_1 - Control	T_8 - KIN 0.5	T_{15} - BAP 1.0 + NAA 1.0	T_{22} - KIN 0.5 + NAA 1.5
T_2 - BAP 0.5	T_9 - KIN 1.0	T_{16} - BAP 1.0 + NAA 1.5	T_{23} - KIN 1.0 + NAA 0.5
T_3 - BAP 1.0	T_{10} - KIN 1.5	T_{17} - BAP 1.5 + NAA 0.5	T_{24} - KIN 1.0 + NAA 1.0
T_4 - BAP 1.5	T_{11} - BAP 0.5 + NAA 0.5	T_{18} - BAP 1.5 + NAA 1.0	T_{25} - KIN 1.0 + NAA 1.5
T_5 - NAA 0.5	T_{12} - BAP 0.5 + NAA 1.0	T_{19} - BAP 1.5 + NAA 1.5	T_{26} - KIN 1.5 + NAA 0.5
T_6 - NAA 1.0	T_{13} - BAP 0.5 + NAA 1.5	T_{20} - KIN 0.5 + NAA 0.5	T_{27} - KIN 1.5 + NAA 1.0
T_7 - NAA 1.5	T_{14} - BAP 1.0 + NAA 0.5	T_{21} - KIN 0.5 + NAA 1.0	T_{28} - KIN 1.5 + NAA 1.5

induction and proliferation studies using different plant growth regulators.

Effect of Various Plant Growth Regulators (PGRs) on Shoot Induction and Proliferation

The response of *E. nuda* to different PGR combinations showed notable variation in shoot initiation and morphogenic development from PLBs. Among all treatments, the combination of BAP (1.0 mg/L) and NAA (0.5 mg/L) (T_{14}) proved most effective, resulting in the earliest shoot initiation (11.20 DAC) as shown in Table 5. At 90 DAC in shooting media the highest number of shoots recorded was 4.60 per explant (Fig. 3) and the maximum shoot length was 4.30 cm (Fig. 4, Plate 3). In contrast, the control treatment showed delayed shoot initiation (38.60 DAC), produced fewer shoots (0.86 per explant) and exhibited a minimum shoot length of 0.40 cm, indicating a weak morphogenic response overall.

The enhanced morphogenic response observed with the BAP (1.0 mg/L) and NAA (0.5 mg/L) combination can be attributed to the synergistic interaction between cytokinin and auxin, which together stimulate both cell division and elongation. Cytokinins such as BAP activate meristematic activity and promote shoot induction, while auxins like NAA facilitate cell enlargement and differentiation, resulting in organized morphogenesis (Dar *et al*., 2021). The balanced BAP:NAA ratio (2:1) provided an optimal hormonal environment that minimized excessive callus formation and developmental abnormalities often associated with cytokinin-dominant media. This equilibrium promoted synchronized shoot initiation and elongation, yielding vigorous and morphologically stable plantlets.

Shoot elongation was most pronounced under the combined treatment, reaffirming the complementary roles of cytokinin and auxin in regulating cell division, expansion and tissue differentiation- consistent with earlier findings in *Hyssopus officinalis* (Shoja and Shishavan, 2021). Elevated cytokinin levels alone can inhibit elongation through ethylene accumulation, emphasizing the importance of maintaining an

TABLE 5

Influence of PGRs and their in combinations on duration for shoot initiation in *E. nuda*

Treatments (mg/L)	Days taken for shoot initiation
T_1 - Control	38.60
T_2 - BAP 0.5	13.80
T_3 - BAP 1.0	12.60
T_4 - BAP 1.5	13.80
T_5 - NAA 0.5	15.20
T_6 - NAA 1.0	15.60
T_7 - NAA 1.5	15.80
T_8 - KIN 0.5	14.60
T_9 - KIN 1.0	14.40
T_{10} - KIN 1.5	13.80
T_{11} - BAP 0.5 + NAA 0.5	13.20
T_{12} - BAP 0.5 + NAA 1.0	13.40
T_{13} - BAP 0.5 + NAA 1.5	13.80
T_{14} - BAP 1.0 + NAA 0.5	11.20
T_{15} - BAP 1.0 + NAA 1.0	12.80
T_{16} - BAP 1.0 + NAA 1.5	15.20
T_{17} - BAP 1.5 + NAA 0.5	14.40
T_{18} - BAP 1.5 + NAA 1.0	14.60
T_{19} - BAP 1.5 + NAA 1.5	14.80
T_{20} - KIN 0.5 + NAA 0.5	13.40
T_{21} - KIN 0.5 + NAA 1.0	13.80
T_{22} - KIN 0.5 + NAA 1.5	14.20
T_{23} - KIN 1.0 + NAA 0.5	14.40
T_{24} - KIN 1.0 + NAA 1.0	13.60
T_{25}- KIN 1.0 + NAA 1.5	14.20
T_{26}- KIN 1.5 + NAA 0.5	13.20
T_{27}- KIN 1.5 + NAA 1.0	13.40
T_{28}- KIN 1.5 + NAA 1.5	13.60
F-Test	**
S. Em.±	0.30
CD at 1%	1.11

** Significant at P= 0.01

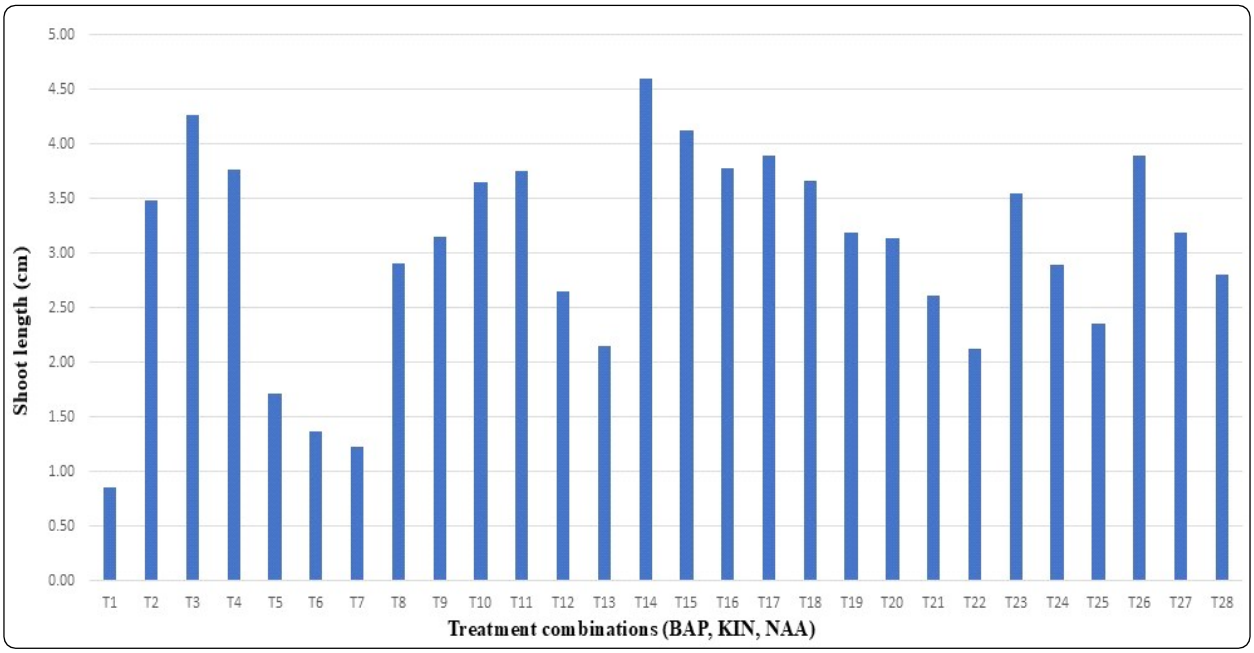

Fig. 4 : Influence of PGRs and their combinationson shoot length (cm) of *E. nuda* at 90 DAC in shooting media

T_1 - Control	T_8 - KIN 0.5	T_{15} - BAP 1.0 + NAA 1.0	T_{22} - KIN 0.5 + NAA 1.5
T_2 – BAP 0.5	T_9 - KIN 1.0	T_{16} - BAP 1.0 + NAA 1.5	T_{23} - KIN 1.0 + NAA 0.5
T_3 - BAP 1.0	T_{10} - KIN 1.5	T_{17} - BAP 1.5 + NAA 0.5	T_{24} - KIN 1.0 + NAA 1.0
T_4 - BAP 1.5	T_{11} - BAP 0.5 + NAA 0.5	T_{18} - BAP 1.5 + NAA 1.0	T_{25} - KIN 1.0 + NAA 1.5
T_5 - NAA 0.5	T_{12} - BAP 0.5 + NAA 1.0	T_{19} - BAP 1.5 + NAA 1.5	T_{26} - KIN 1.5 + NAA 0.5
T_6 - NAA 1.0	T_{13} - BAP 0.5 + NAA 1.5	T_{20} - KIN 0.5 + NAA 0.5	T_{27} - KIN 1.5 + NAA 1.0
T_7 - NAA 1.5	T_{14} - BAP 1.0 + NAA 0.5	T_{21} - KIN 0.5 + NAA 1.0	T_{28} - KIN 1.5 + NAA 1.5

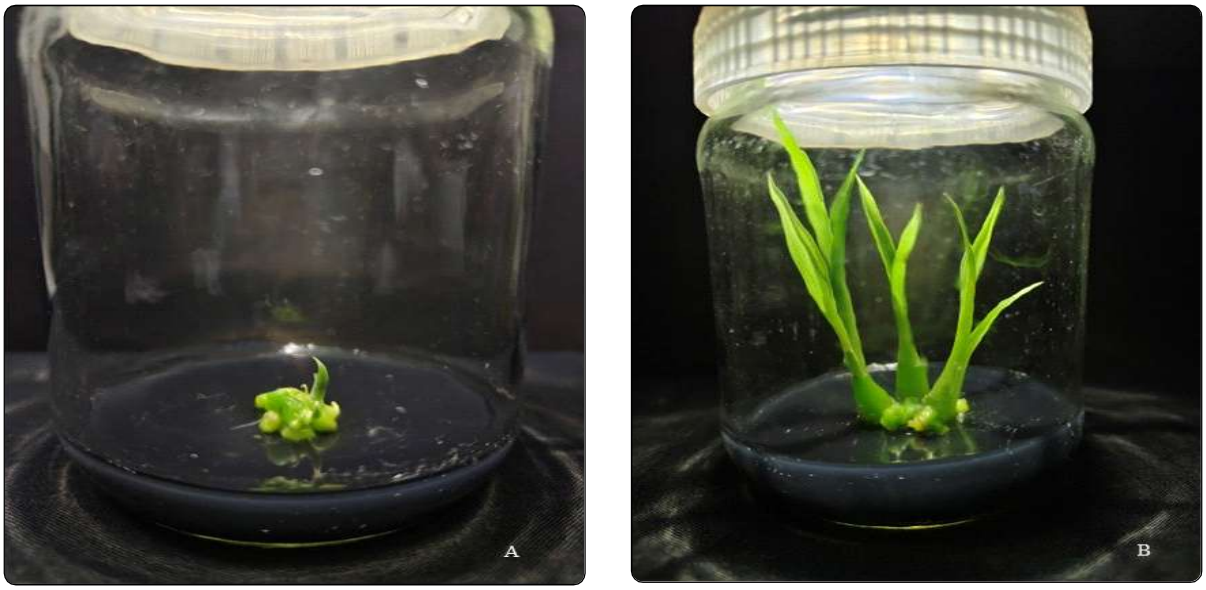

Plate 3 : Shoot proliferation from PLBs in Orchimax full strength at 90 DAC.

(A) In control and (B) BAP (1.0 mg/ L) + NAA (0.5 mg/ L)

appropriate cytokinin: auxin balance (Pasternak and Steinmacher, 2024). Comparable synergistic effects of BAP and NAA have been reported in several orchid species, where their combined application enhanced multiple shoot formation and elongation compared to individual treatments, including *Aerides multiflora* (Bhowmik and Rahman, 2020b) and *Bulbophyllum crassipes* (Ayesha and Islam, 2024). In *E. nuda*, this hormonal combination proved most effective, producing early and uniform shoot initiation, rapid proliferation and robust shoot development. These results underscore the pivotal role of cytokinin: auxin balance in optimizing *in vitro* morphogenesis and achieving the regeneration of healthy, clonal plantlets.

This optimized *in vitro* regeneration protocol represents a significant advancement in the *ex situ* conservation of *E. nuda*, providing a rapid, reproducible and highly efficient system for large-scale propagation through PLBs. Orchimax full strength medium proved most effective for early seed germination, PLB formation and establishment, while the combination of 1.0 mg/L BAP and 0.5 mg/L NAA consistently promoted rapid and uniform shoot initiation, enhanced multiplication and vigorous shoot development. Although proprietary media like Orchimax yield superior results, their cost may limit applicability in resource-constrained settings; therefore, future research should prioritize the development of cost-effective media formulations and the integration of beneficial mycorrhizal fungi during post-PLB stages to improve acclimatization, field establishment and ecological resilience. Overall, the standardized protocol developed in this study provides a robust foundation for mass propagation, restoration programmes, germplasm conservation and the long-term survival of *E. nuda* in its natural habitats.

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Challenges and Strategic Pathways for Strengthening Third Sector Organisations in Karnataka

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Abstract

The present study examined challenges and strategic pathways for strengthening Third Sector Organisations (TSOs) in Karnataka. A total of 30 TSOs-covering NGOs, SHGs and primary-level cooperatives-were selected from Kolar, Chikkaballapur and Tumakuru districts. Garrett's ranking technique was employed to assess the key issues and challenges faced by these organisations. The results revealed the major constraints for NGOs to be, financial limitations (62.33), challenges in reaching target groups (53.87) and women empowerment initiatives (51.13). For PACS, the leading challenges were high overdue and NPAs (66), political interference (64) and lack of infrastructure (36.8), while SHGs primarily reported financial difficulties. Across all TSOs, a heavy dependence on external financial agencies (100%) was observed. The study further highlights that NGOs emphasized diversification of funds and improved governance, SHGs prioritized entrepreneurship and timely credit, while PACS focused on strengthening loan recovery and reducing political interference. These strategies underscore the critical need for financial sustainability, capacity building and institutional reforms to enhance the long-term effectiveness of TSOs in Karnataka. The study recommends strategic financial management, diversification of funding sources and reforms to streamline regulatory processes for strengthening the third sector.

Keywords : TSOs, NGOs, SHGs, Primary level cooperatives

Third sector organisation means a value-driven, non-governmental organization that principally reinvests its surplus funds to further social, environmental or cultural objectives and encompasses voluntary and community organizations, charities, social enterprises, co-operatives and mutual. (Salamon & Anheier, 1998 and Defourny & Nyssens, 2010).

The third sector faces major governance challenges, especially for organizations involved in political or community development activities. They must balance accountability, transparency and stakeholder expectations while pursuing social goals. Increasing market-driven government funding has reduced resources for advocacy-based organizations and partnership networks often threaten the independence of not-for-profits (Barrekat, 2008). NGOs face two main types of challenges: intra-organizational issues such as funding, leadership and management and inter-organizational issues like coordination and public participation. Common problems include inadequate funds, weak leadership, poor communication and urban-centered operations (Ali, 2011 and Kakumani & Kotte, 2011).

NGOs encounter several challenges, including weak institutional structures, lack of transparency, inadequate funding, poor governance and ineffective communication. Many also face political interference,

limited capacity and confusion over regulatory roles. Strengthening leadership, governance and local resource mobilization-along with better use of ICT-can enhance their effectiveness (Prabhakar, 2014). Leadership in the third sector functions in a dynamic social, economic and political environment. It faces pressure to adapt while balancing external challenges with internal issues such as funding stability, organizational independence and commitment to core values (Julie, 2017).

Local participation in NGO activities remains low due to several factors and challenges. Limited community awareness about educational initiatives and NGO programs often hinders effective participation and project success. Additionally, poverty restricts people's involvement in such activities (Anthony, 2020). Funding is another major operational challenge, as many NGOs depend on external and internal institutions, community groups and individuals for financial and moral support (Prasad, 2021).

NGOs face persistent challenges in securing adequate and consistent funding for their voluntary services. Accessing donors and meeting funding conditions remain difficult tasks. Although legally constituted, NGOs may or may not receive government support-some are fully funded by the government, while others receive only partial assistance (Sumanth, 2021).

Another key challenge is 'Mission Creep,' where changing funding trends and external pressures influence organizational goals, diverting NGOs from their original mission. While an NGO's mission provides direction and purpose, shifting donor priorities and structural constraints often make it difficult to remain fully aligned with founding objectives (Liz, 2022).

The third sector in India represents a diverse public space where social aspirations intersect through formal and informal organizations. Cooperatives and NGOs are its most structured forms, while SHGs and community groups extend its grassroots reach. Together, they address developmental challenges and promote socio-economic progress (Rajendran, 2003).

A strong NGO-government relationship depends on shared social objectives and mutual respect. When governments are repressive or weak in poverty alleviation, NGOs often act independently. However, collaborative partnerships can thrive where government policies and NGO goals align, emphasizing autonomy, diversity and genuine cooperation to enhance service delivery (Yunong *et al.,* 2011 and Ani *et al.,* 2014).

NGOs should promote new technologies to boost farm productivity and help the poor acquire assets in a supportive environment. Government assistance in institutional development can strengthen NGOs' role in poverty alleviation, while NGOs should also diversify funding through community savings and private donations rather than relying solely on government support (Muhammad *et al.,* 2011).

Women's empowerment can be achieved through social and economic interventions that enhance competence, self-reliance and self-esteem. These factors contribute to better mental and physical health. Moreover, by strategically engaging with formal and alternative power structures, women can work toward achieving equal rights with men (Robert *et al.,* 2015).

India is home to nearly 3.4 million NGOs, equating to one NGO for every 400 citizens. According to NITI Aayog, in 2020, there were 117,147 registered NGOs across the country, with 53 per cent of them based in rural areas and the remainder in urban settings (https://indianexpress.com). Karnataka alone has 8,322 NGOs dedicated to various social welfare initiatives (https://ngodarpan.gov.in). Additionally, recent estimates show that approximately 40,295 Anganwadi workers, 561 NGOs, 8 Regional Rural Banks, 20 District Central Co-operative Banks and numerous commercial banks are actively involved in promoting Self-Help Groups (SHGs). Collectively, they have helped establish nearly 195,000 SHGs in the state (https://dwcd.karnataka.gov.in).

In Karnataka, out of 5,546 Primary Agricultural Credit Societies (PACS), 5,355 are operational and affiliated with 21 District Central Cooperative Banks (DCCBs), together operating 744 branches. These banks receive refinance from NABARD through the Karnataka State Co-operative Apex Bank. The average loan per farmer is Rs.45,008 and per PACS is Rs.1.80 crore (Dyavanaika and Mokshapathy, 2019). Several third sector organizations, including NGOs, SHGs and primary cooperatives, contribute significantly to rural development and poverty alleviation. Hence, the present study focuses on Kolar, Chikkaballapur and Tumkur districts to examine the link between third sector organizations and government agencies in delivering services to the rural poor.

Methodology

Study Area

The present study was carried out in Kolar, Chickballapur and Tumkur Districts of Karnataka. Three districts were purposely selected for the study due to the presence of a large number of Third Sector Organisations (TSOs) in all three districts.

Sampling Framework

A total of 30 Third Sector Organizations (TSOs) were selected from three districts, comprising 15 Non-Governmental Organizations (NGOs), 10 Self-Help Groups (SHGs) and 5 Primary-Level Cooperatives. Primary data were collected from these TSOs and to meet the study's specific objectives, percentage analysis and Garrett's ranking technique were employed. The data pertain to the year 2021–22.

Garrett's Ranking Technique

Garrett's ranking technique was used to rank the challenges faced by TSOs. The procedure was given by Henry Garrett and Woodworth (1969) suggested the following formula:

$$\text{Per cent position} = \frac{100^* (Rij - 0.5)}{Nj}$$

Where,

Rij = Rank given for i^{th} item by a j^{th} individual

Nj = Number of items ranked by j^{th} individual

The percent position of each rank was converted into scores by referring to Garrett table. Then for each factor, the scores of individual respondents were summed up and divided by the total number of respondents for whom scores were gathered. The mean scores for all the factors were ranked; following the decision criteria that higher the value, more important is the challenge of TSOs.

Results and Discussion

Activities of TSOs (Third Sector Organisations)

Data presented in Table 1 shows that different activities carried out by sample TSOs. All the 30 (100.00%) TSOs are involved in the women empowerment followed by, livelihoods development (76.67%), education (60.00%), agriculture (56.67%), environment (23.33%) and only 6.67 per cent engaged in local governance.

In this study the types of women empowerment have been classified into: Economic empowerment which includes providing training for income generating activities like tailoring and embroidery skills (30-45 days), agarabathi making (5-10 days), agricultural production and livestock activities etc; Social empowerment which includes personal and menstrual hygiene practices and various other community awareness programs and Political empowerment which includes strengthening elected women's leadership & political participation. The education which includes tuition facility for school and college students, skill development programs for graduates, training on job opportunities, quality education upliftment in Govt. schools and awareness on children right etc. Livelihoods development which includes, capacity building, mitigating food insecurity, securing social entitlements, enabling income opportunities through MGNREGS, socioeconomic upliftment of poor, vulnerable and disadvantaged sections of the

community by providing them basic education, trainings, awareness, land development and livelihood interventions, creating awareness, well-being of migrant workers, health and sanitation. Environment which include natural resource management, ecological restoration, sustainable use of natural resources, conservation of biodiversity. The local governance which involves as forests, pastures and water bodies are shared by human settlements and require inter-village cooperation, NGOs works with contiguous villages and traditional forms of local self-governance institutions and nests them within Panchayats to gain formal recognition, to create the institutional apparatus for debate and dialogue at a block or landscape level. They assist rural communities by Reviving rules and regulations for the governance of shared natural resources, helping secure legal rights to access and manage their commons, creating a platform where districts and states recognize and address local needs and agriculture which include organic farming, sustainable agriculture, watershed development, soil testing, providing agri. inputs like seeds for experimental plots, agricultural loan facilities, livestock development, renewable energy, water management and bio-fuel development.

Table 1
Activities of TSO

(n=30)

Activities	No. of TSOs	Percentage
Women empowerment	30 *	100.00
Education	18 *	60.00
Livelihoods development	23 *	76.66
Environment	7 *	23.33
Local governance	2 *	6.67
Agriculture	17 *	56.67

*Multiple activities performed by individual NGOs

Challenges Faced by NGOs

The major constraints faced by NGOs in the study area are shown in Table 2. The study revealed that the major constraints faced by NGOs are financial constraints, followed by challenges in meeting target groups and women empowerment, recording Garrett's scores of 62.33, 53.87 and 51.13 respectively. The major challenge faced by NGOs is related to finance because many NGOs are dependent on external source of funds for organizational activity. Most NGOs reported that the most difficult aspect of successfully running their organisation was raising funds. The activities of an NGO are often influenced by the priorities and preferences of its financiers. Due to this, if an NGO rely on limited sources of funding, they face several difficulties if their funders decide to go on a different route in the given year. Thus, it might be difficult for TSOs to establish a business strategy. One of the most prevalent criticisms levelled against non-governmental organisations is that they should be handled more professionally, like for-profit corporations. However, the difference between the two is that for-profit corporations can make predictions about their profits and revenue sources and typically anticipate where they are going. This is hard for NGOs to accomplish since they rely on donations, grants, contributions for the most part. The second constraint is Challenges in meeting target groups. It was due to NGOs serving at the grassroots level to reach the poor people. The third major

Table 2
Challenges faced by NGOs

(n=15)

Challenges	Garrett's Score	Garrett's Rank
Financial constraints	62.33	I
Administration management	50.33	IV
HR management	43.07	VI
External factors - Social/ Society, Political interferences	50.27	V
Challenges in meeting target groups	53.87	II
Women empowerment	51.13	III

challenge is women empowerment it is because many NGOs are working on women empowerment and they are taking it as a more challenge in the upliftment of women.

The other major challenges were Administration management, External factors - Social/ Society, Political interferences and HR management. NGOs face significant administrative challenges, with poor governance being a major issue. The lack of proper strategic planning exacerbates many of these problems, preventing NGOs from taking ownership of their mission, values and project activities. This vulnerability makes NGOs susceptible to the changing priorities of donors, complicating efforts to measure their long-term impact. In addition, ineffective networking and communication systems hinder collaboration, leading to duplicated efforts, conflicting strategies and a failure to learn from past experiences. Weak networking also breeds suspicion among NGOs and the public, creating a lack of transparency in their operations. Furthermore, political interference in the daily activities of NGOs often disrupts the implementation of developmental projects in specific regions.

The human resource management challenges faced by NGOs stem from the short-term nature of projects and the low priority given to investing in HR capacity building and employee retention. This creates difficulties in retaining skilled, trained employees, as many organizations lack proper HR guidelines to effectively manage human resources. For project-oriented NGOs, high and erratic employee turnover significantly disrupts the implementation of programs. Additionally, employees are often recruited for positions without the necessary qualifications or skills, which negatively impacts their performance and the overall success of the organization. NGOs also struggle to offer competitive incentives and benefits due to financial constraints, further exacerbating retention issues. Many NGOs, especially in India, operate on project-based funding with limited career development opportunities for employees. The uncertainty surrounding funding cycles makes it difficult for organizations to invest in long-term employee growth and capacity development, posing a significant challenge to both the workforce and the organization's effectiveness.

NGOs emphasized financial sustainability as their core concern, prioritizing diversification of funding sources (73.33%) and stronger networking with government programs (80%). They also highlighted the need for improved governance and transparency (66.67%), capacity building of staff and volunteers (60%) and promotion of women's leadership (53.33%). These strategies, if adopted, can reduce donor dependence, enhance professionalism and strengthen grassroots impact.

Challenges Faced by SHGs

The study identifies several major challenges faced by Self-Help Groups (SHGs) in the area, as presented in Table 3. The primary challenges, ranked according to Garrett's scores, are financial difficulties (69.8), followed by training and capacity building (54.3), entrepreneurial activity and group dynamics (39.8) and socio-structural limitations (35.5). The financial challenge stands out as the most significant issue for SHGs, despite their role in enhancing the credit worthiness of women. Concerns about the sustainability of SHGs, particularly their transition from microcredit units to microenterprises, are prominent. Factors affecting their sustainability

TABLE 3

Suggested Strategies by NGOs to Overcome Challenges

(n=15)

Strategies Suggested	No. of NGOs	Per centage (%)
Diversification of funding sources (CSR, local donations, crowd funding)	11	73.33
Capacity building for staff and volunteers	9	60.00
Improved governance and transparency	10	66.67
Networking with government programs and other NGOs	12	80.00
Promoting women's leadership in projects	8	53.33

include poor record-keeping of transactions, lack of mechanisms for ensuring repayment and the inability of some members to settle overdue amounts due to financial hardship. Additionally, SHGs face challenges such as inadequate bank loans, delays in loan disbursement, lack of insurance and poor investment choices. In some cases, bank loans lead to overburdening and malnutrition among members. SHG federations also struggle with poor financial management skills, resulting in low levels of financial sustainability.

The second major challenge faced by SHGs is capacity-building limitations. While access to financial services can significantly enhance the economic productivity and social well-being of poor women, it does not inherently lead to empowerment. To achieve financial self-reliance and successfully use credit for their own enterprises, SHG members require proper training. The third challenge pertains to entrepreneurial activity. One of the core objectives of the SHG system is to empower women both socio-economically and politically, with managerial control over loans being a key indicator of empowerment. Lastly, socio-structural limitations present a significant barrier. In India, family and caste play a critical role in shaping social life and SHGs are no exception. Members often need support from their families to carry out SHG-related tasks such as income-generating activities, bank transactions and attending SHG meetings. In some cases, women rely on family members to manage household chores, particularly when urgent SHG work arises. Moreover, women from upper-caste families face additional challenges, as working outside the home can lead to accusations of bringing dishonour to their families.

The suggestions given by the farm women respondents in Table 3, reports that Farmers Field School (FFS) awareness can be created through SHGs (85.00%) (Yavana *et al.,* 2011).

SHGs prioritized entrepreneurship and skills training (80%) and timely access to credit (70%), reflecting their shift from microcredit to sustainable microenterprises. They also stressed financial literacy (60%), federation for collective bargaining (60%) and family/community support (50%) to overcome socio-cultural barriers. These strategies highlight the dual need for financial empowerment and institutional strengthening to enhance SHG effectiveness.

Challenges Faced by PACS

The major challenges faced by PACS in the study area are shown in Table 4. The study has revealed that the major challenges faced by PACS to be, high overdues and non-performing assets (NPAs) followed by political interferences and lack of necessary infrastructure, recording Garrett's scores of 66, 64 and 36.8 respectively. The major challenge by PACS is High overdue and NPAs because Large over-dues have become a big problem for the PACS. They check the circulation of loanable funds, reduce the borrowing as well as lending power of societies and give them the bad image of the societies of defaulting debtors. Government control over PACS created a moral hazard in lending as a result of which recovery performance suffered severely and thus, PACS over the years, has accumulated the high NPAs. The second major Challenge by PACS is political interferences with dual control of state government and NABARD, PACS are overly excessively occupied and controlled by politicians and bureaucrats which hampers its decision-making and thus compromises corporate governance. The third major challenge is the lack of necessary infrastructure. It is because one of PACS also lacked the necessary digital infrastructure, godown facilities, office facility and trained human resources, still following the old

Table 4

Challenges faced by SHGs

(n=10)

Challenges	Garrett's Score	Garrett's Rank
Financial challenge	69.8	I
Training & Capacity building	54.3	II
Entrepreneurial activity	39.8	III
Socio-structural limitations	35.5	IV

Table 5

Suggested Strategies by SHGs to Overcome Challenges

(n=10)

Strategies Suggested	No. of SHGs	Percentage (%)
Access to adequate and timely credit from banks	7	70.00
Training in financial literacy & book keeping	6	60.00
Entrepreneurship & skills training (tailoring, food processing, etc.)	8	80.00
Strengthening family & community support systems	5	50.00
Federation of SHGs for collective bargaining & sustainability	6	60.00

practice of manual record keeping which not only increases the workload of employees but also created a problem in adopting the latest technology.

PACS strategies focused on strengthening loan recovery (80%), reducing political interference (60%) and modernization through digitalization (60%) and infrastructure development (40%). They also stressed staff training in cooperative management (60%) to address HR gaps. Collectively, these strategies highlight the need for institutional reforms, technological upgrades and capacity building to improve PACS efficiency in rural credit delivery.

Table 6

Challenges faced by PACS

(n=5)

Challenges	Garrett's Score	Garrett's Rank
Political interferences	64	II
High overdue and NPAs	66	I
Other Linked Inputs, Extension Service and Marketing	36.8	IV
Digitalization	27.2	V
Poor infrastructure facilities	55	III

Major Financial Challenges Faced by TSOs

The major financial challenges faced by TSOs in the study area are shown in Table 5. The study has revealed that cent per cent of respondents depended on external financial agencies. All observed entities seem to rely on external financial agencies for funding. While external funding can provide necessary capital, over-reliance on it may pose risks such as high interest rates, stringent terms and conditions or vulnerability to changes in external funding sources. Next financial challenge faced was Limitations on Raising Funds from Government and Other Financial Institutions (83.33%). This suggests that a majority of entities encounter barriers or restrictions when attempting to secure funds from government bodies or traditional financial institutions. These limitations could be due to bureaucratic processes, eligibility criteria or competition for available funds. Inadequate Financial provision was reported by 66.67 per cent of

Table 7

Suggested Strategies by PACS to Overcome Challenges

(n=5)

Strategies Suggested	No. of PACS	Percentage (%)
Strengthening loan recovery mechanisms	4	80.00
Reducing political interference through policy reforms	3	60.00
Investment in digitalization and record automation	3	60.00
Infrastructure development (storage, office facilities)	2	40.00
Training staff in modern cooperative management	3	60.00

TABLE 8

Major financial problems faced by TSOs

(n=30)

Financial Problems	No. of TSOs	Percentage
Inadequate Financial Provision	20 *	66.67
Dependence on external financial agencies	30 *	100
Limitations on raising funds from government and other financial institutions	25 *	83.33
The complicated legal process in getting foreign funding	10 *	33.33

*Multiple responses

respondents. This indicates that a significant portion of the observed entities face issues due to insufficient financial resources allocated to them. This could lead to various challenges such as inability to meet operational expenses, invest in growth opportunities, or sustain existing projects. Complicated Legal Process in Getting Foreign Funding (33.33%) was the least challenge. While not as prevalent as other issues, a significant proportion of observed entities face challenges related to the legal complexities involved in securing foreign funding. This could include compliance with international regulations, navigating foreign legal systems, or dealing with currency exchange issues.

Overall, the data highlights a range of financial challenges faced by the entities under observation, emphasizing the need for strategic financial management, diversification of funding sources and potentially reforms to streamline legal and regulatory processes.

The recovery rate from large farmers showed a significant growth, with a 1 per cent increase in irrigated areas and a 5 per cent increase in dryland areas. However, the loan recovery rate from large farmers was lower compared to small farmers. This discrepancy can be attributed to the higher expectations among large farmers regarding government loan waiver schemes (Guruprasad and Umesh, 2015).

The findings underscore that the financial challenges are pervasive across various types of organizations, including NGOs, Self-Help Groups (SHGs), Primary Agricultural Credit Societies (PACS) and other third sector organizations (TSOs). These challenges range from inadequate financial provision and dependence on external financial agencies to limitations on raising funds from government sources and navigating complicated legal processes for foreign funding. The consistent presence of these challenges underscores the critical need for strategic financial management, diversification of funding sources and potential reforms to streamline regulatory processes.

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SSR Marker-Based Evaluation of Genetic Variability in Coorg Mandarin (*Citrus reticulata* Blanco) Clones

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Abstract

Coorg mandarin, a GI-tagged crop native to Kodagu district of Karnataka, is renowned for its rich flavor resulting from a fine balance of sugars and acids, along with good keeping quality. Coorg mandarin being polyembryonic, most orchards consist of seedling-origin trees, hinting at inherent genetic diversity in the region. To assess this diversity, 11 SSR markers were used on 49 Coorg mandarin accessions. Among these, CMS4 and TAA33 exhibited higher polymorphism (0.604 and 0.492), while TAA41, CAC23 and TAA52 exhibited moderate PIC (0.25–0.5). Amplified product sizes ranged from 80 to 300 bp, with CAC19 and TAA15 showing the broadest amplification. Shannon's Information Index (I) ranged from 0.000 to 0.686, highest for TAA33 (0.686) and CMS4 (0.680). Genetic distances between accessions varied from 0.00 to 0.90. Close genetic similarity (0.00) was observed between Kottageri and Kattemadu; also, Sulugod and Kuttandi, which suggests the clonal origin or recent divergence. The highest divergence (0.90) was seen between Kanoor and Srimangala. A dendrogram constructed using Nei's genetic distance matrix and UPGMA clustering revealed two major clusters. The clones Kanoor and Srimangala were out grouped indicating a unique genetic identity, possibly from geographic isolation or ecological adaptation. Other clusters comprising Boikeri, Perur, Sulugod and Kuttandi reflected low divergence and were closely related which may indicate shared regional gene pool and similar environmental conditions.

Keywords : GI-tagged, SSR markers, Nei's genetic distance, Coorg mandarin

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The mandarins (*Citrus reticulata* Blanco), are one of the core citrus taxa. The exact location of origin of mandarins is unknown, it is believed to be a native of south eastern Asia and the Philippines and might have evolved in a region including Vietnam, South China and Japan. It is one of the important citrus fruits that is grown in India. There are three distinct ecotypes of mandarin in India, the Nagpur mandarins, the Coorg mandarins and the Khasi mandarins (Tripathi, 2023).

The Coorg mandarin (*Citrus reticulata* Blanco) is a famous ecotype of mandarin grown in the agro-ecology of the region over a century in South India. Coorg mandarins are known to be grown in hilly areas and elevation of up to 1700 m in Coorg region and spread in other parts of Western Ghats such as Waynad, Palni and Sevroy hills, Hassan and Chikkamagalur areas (Tripathi and Karunakaran, 2013). Coorg mandarin is grown in a multi-tier cropping system as an intercrop with coffee and pepper. This cropping system has proved efficient with higher land use efficiency. In 2013 and 2014 the Coorg mandarin was cultivated in an area of 1,569 hectares with a production of 39,225 tonnes (Bopanna *et al*., 2016). Later on, there is decline in the area and production of Coorg mandarin and reached to all time

low in year 2000 (Tripathi and Karunakaran, 2013). The exact data of area and production in Coorg mandarin is not available at the present scenario.

By the 1980s, the orange crop in the Coorg district of Karnataka was primarily affected due to an outbreak of greening disease, commonly referred to as citrus decline. This issue has brought down the cultivation of the crop to a larger extent. When the problem persisted, the only remedy was to go for mass destruction of the crop and replant new saplings. Mass destruction was carried out by the growers but the replacement rate was very poor (Hemanthkumar, 2001). The other reason contributing to the decline in the area of cultivation is the difficulty in managing the crop alongside Coffee, which is a major commercial crop in the district of Kodagu. The lower market price, shortage of quality planting material, infestation of Loranthus (Partially parasitic plant), fruit drop followed by a lengthy list of pest and diseases caused by various organisms are the main reasons for the sharp decline in the Coorg mandarin production in the region over years. Genetically superior, disease-free propagating material is the foundation stone for a successful scientific citrus cultivation. In order to pursue the possibility of identifying and selecting superior clones, targeted surveys can be a continued process in the traditional growing regions to exploit the existing potential.

A way to access the genetic diversity in a population is by the use of markers. Markers are any trait of an organism can be identified with confidence and relative ease. There are different types of markers *viz.,* morphological, biochemical and DNA based molecular markers (Genetic marker). A genetic marker is a gene or DNA sequence with a known location on a chromosome and associated with a particular gene or trait (Al-Samarai and Al-Kazaz, 2015). Over the last few decades, the use of molecular markers to identify polymorphism at the DNA level has been increasingly important in plant biotechnology and genetic research. Among the different DNA based markers such as, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), sequence-tagged sites (STSs) etc., SSRs are the most widely used markers for the diversity studies. The simple sequence repeats (SSR) markers are highly reproducible due to their primer length and the high stringency achieved by the annealing temperature and provides highly polymorphic finger prints (Kumar *et al.,* 2021). The clones or accessions selected through targeted surveys if subjected to molecular characterization, will give us the idea about the existing diversity of the crop and hence this study was conducted with an objective to assess the genetic diversity of Coorg mandarin clones using SSR markers.

Material and Methods

The leaf samples from Coorg mandarin (*Citrus reticulata* Blanco) clones (Table 1) were collected and the genomic DNA was isolated using CTAB method (Doyle and Doyle, 1987) with slight modifications. Young and healthy leaves (2 g) which were collected from the field were cleaned and crushed using the tissue lyser by adding 900 µL of pre-warmed 1.5 per cent (W/V) CTAB buffer. The samples were incubated for 60 min. at 65 °C in a water bath and centrifuged for 10 minutes at 12000 RPM. The supernatant was transferred into another Eppendorf tube where equal

Table 1

List of Coorg mandarin accessions collected from Kodagu district

Name of the accession	Name of the accession
Kottageri 3	Ponnapsanthe 6
Kottageri 4	Ponnapsanthe 7
Sulugod 1	Arecad 3
Sulugod 3	Arecad 4
Sulugod 4	Ammangala 3
Sulugod 5	Ammangala 4
Sulugod 6	Nanjarayapatna 2
Sulugod 7	Perur 2
Sulugod 8	Perur 3
Sulugod 10	Perur 5
Devanoor	Kattemadu 1

Continued....

Table 1 Continued....

Name of the accession	Name of the accession
Srimangala	Kattemadu 2
Kanoor	Kattemadu 3
Sirangalli 4	Konankatte 1
Mallur 1	Konankatte 3
Mallur 2	Konankatte 4
Mallur 4	Konankatte 5
Kuttandi 1	Rajapura 1
Kuttandi 2	Rajapura 2
Boikeri	Rajapura 3
Mayamudi 1	Rajapura 4
Mayamudi 2	Rajapura 5
Ponnapsanthe 2	Nittur 1
Ponnapsanthe 3	Nittur 2
Ponnapsanthe 5	

volume of chloroform: Isoamyl alcohol mixture (24:1 v/v) was added and mixed by inversion. It was then centrifuged at 12000 RPM at 4 °C for 10 minutes. This step was repeated twice to ensure removal of protein contaminants. The supernatant was transferred to a new tube. Equal volume of ice cold isopropanol was added and mixed gently by inversion and then kept in the freezer (-20 °C) overnight for precipitation. The next day, the samples were centrifuged at 12000 RPM for 10 minutes. The pellet was washed with 50-100 µL of 70 per cent ethanol and again centrifuged for 10 minutes. The DNA pellet was air dried for 4-5 hours after removing the supernatant. According to the size of the DNA pellet, 30 to 200 µL of TE buffer (pH 8) was added to dissolve the DNA and kept in freezer at -20 °C for further use.

A total of 12 SSR primer pairs (Table 2) were used for the PCR reaction. Each reaction was set up in a final volume of 10µL. Takara master mix (2X) (Taq DNA polymerase, dNTPs (0.4 mM each at 1× concentration), 1.5-2.0 mM$MgCl_2$, reaction buffer, stabilizers and loading/tracking dyes) of 5.0 µL, forward and reverse primer each of 0.5 µL, 1.0 µL of Template DNA (10-50 ng/µL) and 3 µL of nuclease free water was used in the reaction. Annealing temperature (Table 2) was standardized for each primer using gradient PCR. Out of the 12 primers used in the study, only 11 primers showed amplification and hence those were considered for further data analysis.

The PCR product size data obtained in base pairs (bp) was used for all the statistical analysis. Observed

Table 2

List of SSR primers and their annealing temperature

SSR marker	Forward	Reverse	Annealing temperature
TAA15 (Aleza *et al.,* 2009)	GAAAGGGTTACTTGACCAGGC	CTTCCCAGCTGCACAAGC	54.1
TAA41 (Haque *et al.*, 2022)	AGGTCTACATTGGCATTGTC	ACATGCAGTGCTATAATGAATG	51.6
TAA33 (Barkley *et al*., 2006)	GGTACTGATAGTACTGCGGCG	GCTAATCGCTACGTCTTCGC	54.1
TAA52 (Kacar *et al.,* 2013)	GATCTTGACTGAACTTAAAG	ATGTATTGTGTTGATAACG	41.4
TAA45 (Kacar *et al.,* 2013)	GCACCTTTTATACCTGACTCGG	TTCAGCATTTGAGTTGGTTACG	54.6
TAA 27 (Barkley *et al*., 2006)	GGA TGA AAA ATG CTC AAA ATG	GAG GGA AAG CCC TAA TCT CA	50.4
TAA3 (Barkley *et al*., 2006)	AGAGAAGAAACATTTGCGGAGC	GAGATGGGACTTGGTTCACACG	53.2
CAC23 (Haque *et al.*, 2022)	GGTGATGCTGCTACTGATGC	CAATTGTGAATTTGTGATTCCG	52.6
CAC19 (Kacar *et al.,* 2013)	ACAACCTTCAACAAAACCTAGG	AAGACTTGGTGCGACAGG	49.7
CMS4 (Barkley *et al*., 2006)	CCTCAAACCTTCTTCCAATCC	CTGTAAAGTACATGCATGTTGG	42.9
ATC09 (Kacar *et al.,* 2013)	TTCCTTATGTAATTGCTCTTTG	TGTGAGTGTTTGTGCGTGTG	50.6
AC01	TTTGACATCAACATAAAACAAGAAA	TTTTAAAATCCCTGACCAGA	-

heterozygosity (Ho), the expected heterozygosity (He), and the polymorphic information content (PIC) were calculated using R 2.0 version software. The cultivars were grouped by cluster analysis using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) algorithm.

Results and Discussion

Genetic Diversity Analysis in Coorg Mandarin Clones Using SSR Markers

A total of 11 SSR (Simple Sequence Repeat) markers data was used to assess the genetic diversity among the selected populations of Coorg mandarin (Table 1). The summary of the characteristics of the primers used in this study, including the number of alleles per locus and the Polymorphic Information Content (PIC), are presented in Table 3.

The higher allelic variation was observed at CAC23 (Fig. 1), which exhibited 3 alleles, indicates a higher degree of polymorphism at this locus. The loci such as TAA15, TAA52, TAA45, TAA 27 and TAA3 displayed only 2 alleles, reflecting relatively lower variability. Ample studies have been done in various citrus species to study the genetic diversity using SSR markers and it was noted that, the genetic relationships and diversity of 51 accessions of sour orange (*Citrus aurantium*) and their relatives using seventeen SSR primers produced a mean of 1.4 allele per marker (Polat *et al.,* 2012). When 12 SSR primers were used to study the genetic variation among 56 accessions (G1~G56) of Citrus in Iran. The lower number of alleles was observed on CAGG9 locus with 2 alleles and the higher number of alleles was observed in TAA41 locus with 8 alleles (Jannati *et al*., 2019).

The Polymorphic Information Content (PIC), a measure of the informativeness of a marker, varied from 0.079 (TAA3) to 0.604 (CMS4). Markers with PIC values greater than 0.5 (e.g., CMS4 and TAA33) are considered highly informative, while those with values between 0.25 and 0.5 (e.g., TAA41, CAC23, TAA52) are moderately informative. Markers with values less than 0.25 (e.g., TAA3, CAC19) are less informative and may contribute less to the assessment of genetic variation. Overall, the mean PIC value across all loci was indicative of moderate informativeness of the SSR markers used in this study. Similarly, Kaur *et al*. (2022) have reported, the Polymorphism information content values ranging from 0.13 (CIBE 5156) to 0.73 (CCSM 40), with an

Table 3

Characteristics of SSR primers used for the diversity study

Primer code	Amplified product range (bp)	Number of bands			Polymorphic information content (PIC) value
		Total	Polymorphic	Monomorphic	
TAA15	200-300	1	0	1	0.193
TAA41	100-220	2	1	1	0.388
TAA33	80-220	2	1	1	0.492
TAA52	150-250	1	0	1	0.265
TAA45	150-250	1	0	1	0.193
TAA 27	80-150	1	0	1	0.366
TAA3	100-200	1	0	1	0.079
CAC23	180-280	3	2	1	0.307
CAC19	150-300	2	1	1	0.135
CMS4	100-250	2	1	1	0.604
ATC09	80-220	2	1	1	0.352

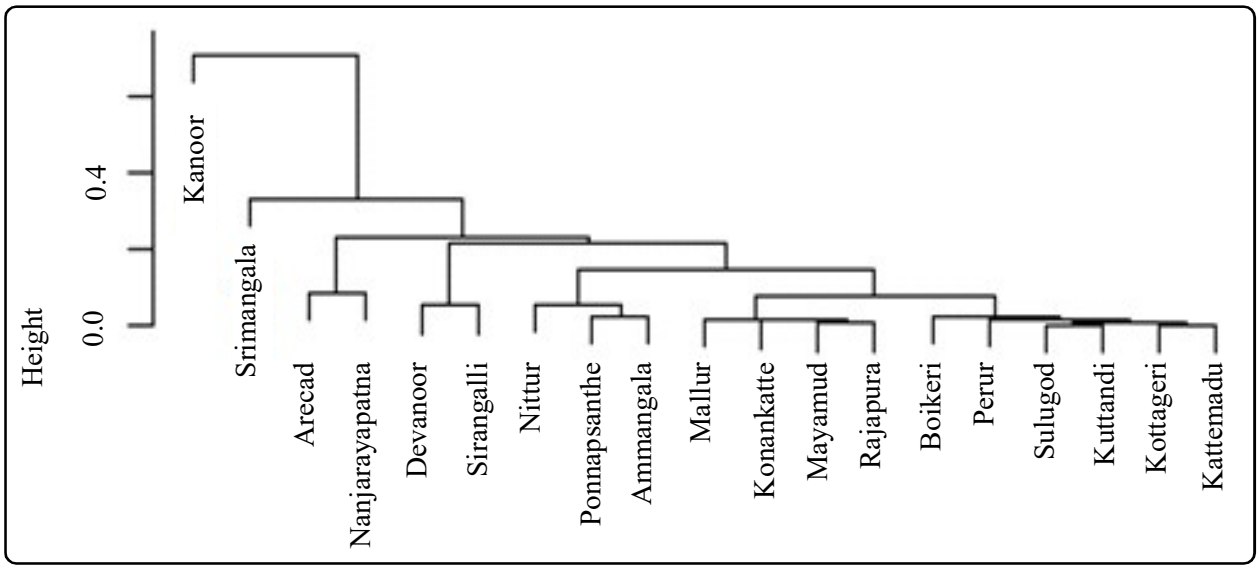

Fig. 2 : Dendrogram showing the genetic relationships among 20 populations based on Nei's genetic distance, constructed using the UPGMA method

(The vertical scale represents genetic distance, with shorter distances indicating greater genetic similarity)

average value of 0.45 across all genotypes of mandarins grown in different countries. In another study by Munankarmi *et al.* (2023), the PIC value ranged from 0.497 with primer TAA 27 to 0.802 with primer TAA 41 with an average value of 0.662, which were used to characterize genetic diversity of forty-five *Citrus* accessions collected from National Citrus Research Program (NCRP), Dhankuta and Kathmandu valley.

The amplified product size range across primers varied from 80 bp to 300 bp. The widest amplification range was observed with CAC19 (150 to 300 bp) and TAA15 (200 to 300 bp), suggesting a broader binding region or possible repeat variation at those loci. In terms of loci amplification, the total number of loci per primer ranged from 1 to 3. Most primers (e.g., TAA15, TAA52, TAA45, TAA 27, TAA3) amplified only a single monomorphic locus, indicating a lack of visible variation across the samples for those loci.

Moderate levels of polymorphism were detected in primers such as TAA41, TAA33, CAC19, CMS4 and ATC09, each producing two bands, with one being polymorphic and the other monomorphic. These primers contributed to identifying variability across the population set and enhanced the overall effectiveness of the marker used in the study. Conversely, primers such as TAA15, TAA52, TAA45, TAA 27 and TAA3 produced exclusively monomorphic bands, offering limited utility in detecting allelic differences among individuals. While these loci may be conserved or fixed across the sampled populations, they contribute little to the assessment of intra-population variation.

Overall, out of the total 18 loci amplified across all primers, 7 loci were polymorphic, while 11 were monomorphic. The average number of polymorphic loci per primer was 0.73, indicates a relatively low but notable level of genetic variability detectable by the SSR markers in this study. In a genetic diversity analysis of 46 mandarins grown in different countries by Kaur *et al*. (2022) showed that, out of 110 SSR markers, 64 polymorphic markers amplified a total of 155 alleles. Similarly, in a study conducted by Rohini *et al.* (2020), thirty-eight accessions of *C. jambhiri* were characterized using 17 SSR markers. A total of 60 loci were generated, of which all the 60 were polymorphic (100%). These findings highlight the varying degrees of effectiveness among SSR primers in revealing polymorphism. Primers such as CAC23, CMS4 and TAA33 were more effective in

detecting genetic variation, whereas primers like TAA15 and TAA3 were less informative due to the absence of polymorphic bands. This variability underscores the importance of carefully selecting highly polymorphic and informative SSR markers for robust genetic diversity assessments.

Genetic Diversity Parameters Across SSR Loci

Several standard diversity indices were calculated, including the number of individuals genotyped (N), observed number of alleles (Na), effective number of alleles (Ne), Shannon's Information Index (I), observed heterozygosity (Ho), expected heterozygosity (He) and unbiased expected heterozygosity (uHe) and are represented in Table 4.

The number of individuals (N) genotyped per locus ranged from 39 (TAA 27) to 49 (TAA33), reflecting minor differences due to potential amplification success or missing data across samples due to non-amplification of certain accessions. The observed number of alleles (Na) per locus ranged from 1 to 3. Six loci (TAA15, TAA52, TAA45, TAA 27, TAA3) exhibited only one allele (Na = 1), indicates a monomorphic nature and thus contributes no allelic variation to the genetic analysis. CAC23 showed the highest allelic richness with three alleles (Na = 3), followed by several loci (TAA41, TAA33, CAC19, CMS4, ATC09) that revealed two alleles, which indicates polymorphism at these loci. The effective number of alleles (Ne), which accounts for both the number and frequency of alleles, ranged from 1.000 to 1.970. Notably, TAA33 (Ne = 1.970) and CMS4 (Ne = 1.948) showed the highest values, suggesting a more even allele distribution and higher diversity at these loci. In contrast, the monomorphic loci had an Ne of 1.000, indicates a lack of allele frequency variation.

Shannon's Information Index (I), a measure of genetic diversity that incorporates both richness and evenness of alleles, ranged from 0.000 to 0.686, with the highest values again observed at TAA33 (0.686) and CMS4 (0.680). The expected heterozygosity (He), which measures the probability that two randomly selected alleles are different in the population, varied significantly across loci, ranging from 0.000 (TAA15, TAA52, TAA45, TAA 27, TAA3) to a maximum of 0.837 (CMS4) (Table 4). A zero value for 'He' suggests complete homozygosity at those loci, indicates either a strong fixation of alleles or limited variability within the populations at those markers.

Table 4

Genetic diversity parameters based on SSR primers studied in 49 Coorg mandarin accessions

Locus	N	Na	Ne	I	Ho	He	uHe
TAA15	44	1.000	1.000	0.000	0.000	0.000	0.000
TAA41	43	2.000	1.259	0.359	0.140	0.206	0.208
TAA33	49	2.000	1.970	0.686	0.796	0.493	0.498
TAA52	42	1.000	1.000	0.000	0.000	0.000	0.000
TAA45	44	1.000	1.000	0.000	0.000	0.000	0.000
TAA 27	39	1.000	1.000	0.000	0.000	0.000	0.000
TAA3	47	1.000	1.000	0.000	0.000	0.000	0.000
CAC23	46	3.000	1.274	0.423	0.196	0.215	0.217
CAC19	48	2.000	1.110	0.205	0.104	0.099	0.100
CMS4	43	2.000	1.948	0.680	0.837	0.487	0.492
ATC09	47	2.000	1.421	0.473	0.362	0.296	0.299

N- total no. of observations; Na- no. of different alleles; Ne- no. of effective alleles; I- Shannon's information index; He- expected heterozygosity; uHe- unbiased heterozygosity

In contrast, observed heterozygosity (Ho), which reflects the actual proportion of heterozygotes in the population, ranged from 0.000 to 0.493, with the highest observed at TAA33 and CMS4 (Table 4). Interestingly, some loci such as TAA41 and CAC23 showed Ho values higher than their corresponding He values, which could suggest an excess of heterozygosity possibly due to non-random mating, selection, or sampling effects.

In support of the present findings on the molecular characterization of Coorg mandarin, a study conducted by Munakarmi *et al*. (2023) on various citrus species in Nepal reported that, the Observed Heterozygosity (Ho) ranged from 0.460 to 0.794 and the Expected Heterozygosity (He) also ranged from 0.460 to 0.794. Additionally, Shannon's Information Index (I) varied between 0.677 and 1.678, indicates considerable genetic diversity among the studied accessions. In wood apple (*Limonia acidissima*) being in the same family of citrus that is rutaceae showed moderate gene diversity (0.226 ± 0.007) and Shannon's information index (0.369 ± 0.009) in the species (Lata *et al.,* 2024).

These results highlight substantial variation in the informativeness of the SSR markers used in this study. TAA33 and CMS4 emerged as the most informative loci, exhibits the highest diversity indices across all parameters. The inclusion of such markers is crucial in accurately capturing the genetic variation within and among populations.

Pairwise Genetic Distance among Coorg Mandarin Accessions

To assess the genetic divergence, the forty-nine accessions were grouped into 19 populations based on the site of collection and Nei's genetic distance was calculated based on SSR marker data for those nineteen populations. The results of pairwise population matrix (Table 5) provides an estimate of the genetic distance between each pair of population group, that reflects how genetically similar or distinct they are from one another.

The genetic distance values in the matrix ranged from 0.00 to 0.90, which indicates considerable variability among the accessions. The lowest genetic distance (0.00) was observed between several pairs of accessions, including Kottageri and Kattemadu; also Sulugod and Kuttandi, that suggests a very close genetic relationship or possible clonal origin. In contrast, the highest genetic distance (0.90) was observed between Kanoor and Srimangala, which reflects a substantial degree of genetic divergence. Further, the Kanoor is distantly related to all other clones in the present study, indicating the possibility of mutation leading to distinct loci compared to others.

A number of accession pairs showed very low genetic distance values (≤ 0.05) (Table 5), such as:

- Kottageri and Sulugod (0.01)
- Sulugod and Kuttandi (0.00)
- Boikeri and Sulugod (0.01)
- Kattemadu and Kuttandi (0.01)
- Rajapura and Mallur (0.01)

These low values suggest a high degree of genetic similarity, possibly due to shared ancestry, gene flow, or limited geographical separation. Such closely related accessions may represent selections from the same population or clonal propagation from a common ancestor.

On the other hand, accessions like Kanoor were consistently more genetically distant from the others. For example:

- Kanoor and Srimangala (0.90)
- Kanoor and Mallur (0.58)
- Kanoor and Kattemadu (0.49)

These high values suggest that, Kanoor represents a genetically distinct lineage, which may have evolved in relative isolation or originated from a different gene pool. Such distinct genotypes could be valuable for broadening the genetic base in breeding programmes.

The moderate to high genetic distances observed in many comparisons support the notion that the Coorg mandarin population is not genetically uniform, but rather exhibits structured diversity across geographic locations. This diversity can be utilized for selective breeding, conservation planning and genetic resource management. The Nei's genetic distance matrix reveals a complex pattern of relatedness among Coorg mandarin accessions, with both closely related clusters and highly divergent genotypes. This analysis

TABLE 5

Pairwise population matrix of Nei genetic distance of Coorg mandarin accessions

	Kottageri	Sulugod	Devanoor	Srimangala	Kanoor	Sirangalli	Mallur	Kuttandi	Boikeri	Mayamud	Ponnapsanthe	Arecad	Ammangala	Nanjarayapatna	Perur	Kattemadu	Konankatte	Rajapura	Nittur
Kottageri	0.00																		
Sulugod	0.01	0.00																	
Devanoor	0.11	0.11	0.00																
Srimangala	0.21	0.21	0.40	0.00															
Kanoor	0.45	0.47	0.29	0.90	0.00														
Sirangalli	0.05	0.05	0.05	0.29	0.35	0.00													
Mallur	0.04	0.03	0.16	0.16	0.58	0.10	0.00												
Kuttandi	0.01	0.00	0.12	0.23	0.49	0.06	0.04	0.00											
Boikeri	0.03	0.01	0.14	0.26	0.54	0.08	0.05	0.01	0.00										
Mayamudi	0.04	0.04	0.17	0.18	0.57	0.10	0.02	0.03	0.04	0.00									
Ponnapsanthe	0.03	0.03	0.15	0.15	0.52	0.07	0.04	0.03	0.05	0.02	0.00								
Arecad	0.05	0.06	0.18	0.31	0.40	0.11	0.10	0.06	0.08	0.10	0.09	0.00							
Ammangala	0.03	0.03	0.14	0.19	0.43	0.08	0.07	0.03	0.05	0.04	0.03	0.08	0.00						
Nanjarayapatna	0.14	0.12	0.22	0.47	0.49	0.14	0.18	0.12	0.12	0.17	0.17	0.09	0.18	0.00					
Perur	0.02	0.01	0.15	0.22	0.55	0.09	0.04	0.02	0.03	0.05	0.05	0.07	0.04	0.15	0.00				
Kattemadu	0.00	0.01	0.13	0.22	0.49	0.07	0.04	0.01	0.03	0.04	0.04	0.06	0.03	0.17	0.01	0.00			
Konankatte	0.02	0.02	0.12	0.19	0.49	0.06	0.02	0.02	0.04	0.02	0.02	0.07	0.04	0.15	0.04	0.02	0.00		
Rajapura	0.03	0.03	0.14	0.17	0.53	0.08	0.01	0.03	0.05	0.01	0.02	0.09	0.05	0.16	0.05	0.04	0.01	0.00	
Nittur	0.12	0.13	0.27	0.14	0.63	0.18	0.11	0.13	0.16	0.07	0.04	0.19	0.06	0.31	0.14	0.12	0.10	0.08	0.00

highlights the presence of genetic substructure within the population. It also supports the potential for genetic improvement through the selection of diverse and complementary parental lines.

The genetic distance varies with the marker used, species and population within the species. This can be supported by various studies conducted by researchers across wide range of crops. For example, low Nei's genetic distance (0.31) among ten genotypes of wood-apple trees which is of the same family as of citrus (rutaceae) sampled from two Indian states, Madhya Pradesh (Ratlam, Indore) and Gujarat (Vadodara) were recorded in a study conducted by Lata *et al*. (2024). Here it can be noticed that the genetic distance between genotypes could be as low as 0.31 even though they were sampled from two different states. Another study in which the Nei's genetic distances were calculated for Khasi mandarin (*Citrus reticulata* Blanco) in Meghalaya using 11 polymorphic SSR locus, revealed varying genetic relationships among locations, with a minimum distance of 0.038 between Umsohme and Sokhwai and a maximum distance of 1.418 between Sokhwai and Umta (Kar *et al*., 2024). The analysis of Nei's genetic distance between siam and keprok citrus populations showed genetic distance value of 0.419, where thirty-seven mandarin genotypes consists of 7 siam cultivars, 28 keprok cultivars and 2 cultivars derived from their hybridization, were subjected to genetic diversity analysis using 20 SSR markers (Nugroho *et al.,* 2025).

Genetic Relationship among Populations Based on Nei's Genetic Distance

A dendrogram was constructed using Nei's genetic distance matrix and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering algorithm (Fig. 2). This analysis provides insights into the extent of genetic similarity and divergence across the populations based on multilocus allele frequency data.

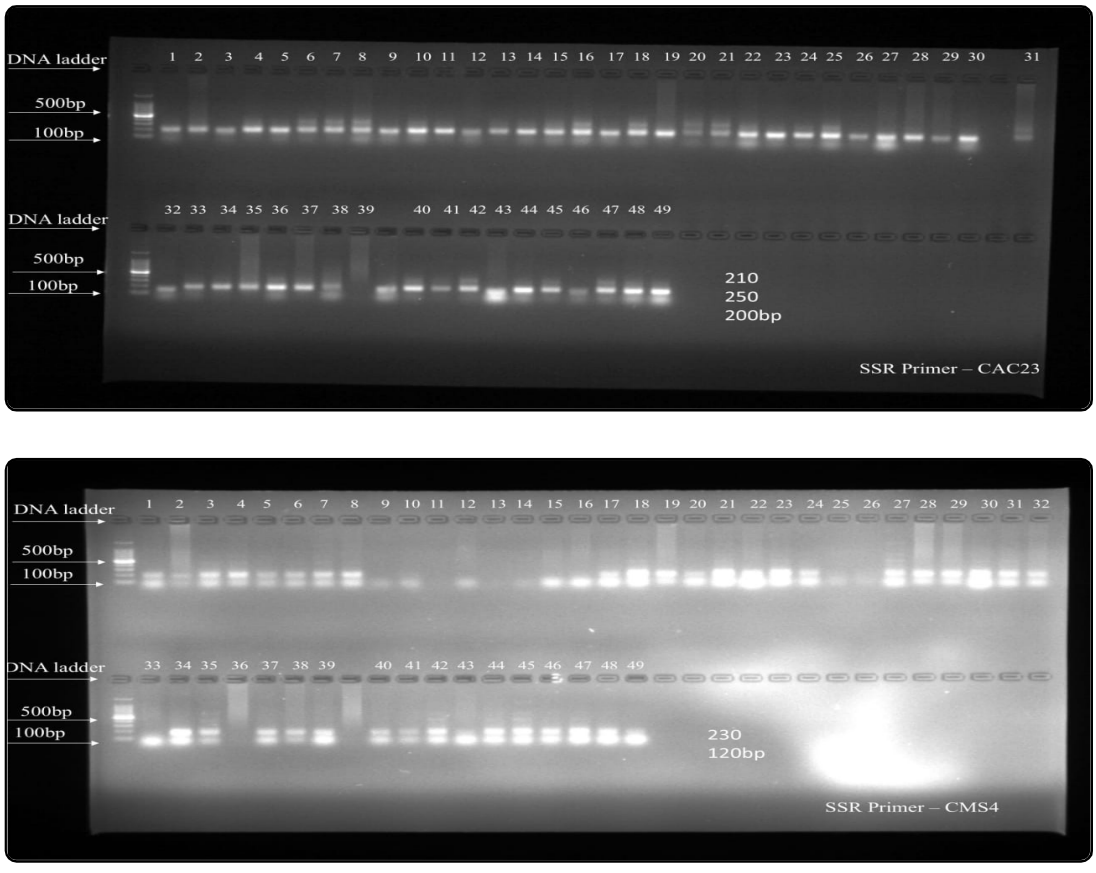

Plate 1 : The Gel documentation of the SSR Primer CAC23 and CMS4 used in the study

The dendrogram revealed several well-defined clusters. Notably, Kanoor was out grouped and it was related to Srimangala and others vary distantly. Whereas the other clones were very closely related. The low height at which these populations join and indicate a minimal genetic differentiation, which influenced by geographic proximity or recent common ancestry. Their early branching pattern, relative to the rest of the populations, also implies a unique genetic identity that may result from prolonged isolation or habitat specificity.

The clones such as Arecad and Nanjarayapatna, Devanoor and Sirangalli, Nittur, Ponnapsanthe and Ammangala. Mallur, Mayamudi, Konankatte and Rajapura were placed in related small clusters indicating their genetic similarity at SSR loci probed in the current study.

A second prominent cluster comprises Boikeri, Perur, Sulugod and Kuttandi, which exhibit low levels of genetic divergence and form a sub-group. The close genetic proximity among these populations could be attributed to share the ecological conditions, or limited barriers to dispersal. Their clustering pattern indicates that, they may form part of a genetically homogeneous regional group.

Populations such as Kattemadu, Kottageri and Kuttandi are also closely related, as reflected in their shallow inter-branch distances. This further supports the presence of regional genetic similarity within this subset, possibly due to overlapping habitats or recent gene exchange.

These groupings may reflect intermediate evolutionary histories, where some gene flow occurs but is limited by ecological or geographical constraints. Interestingly, the clustering pattern shows that, Kanoor and Srimangala, despite being very similar to each other and are genetically more distant from the remaining populations. This is evidenced by their separation from other groups at a relatively higher height on the dendrogram. Overall, the dendrogram highlights clear genetic structuring among the sampled populations.

In a phylogenetic analysis of Indonesia's mandarin genotypes using SSR markers clearly revealed separation between siam and keprok group. Since there was no clear differentiation of citrus genotypes by geographic origin, the clustering pattern observed in a study was based on the genetic background variation (Nugroho *et al*., 2025). Clustering based on UPGMA algorithm for morphological data were analyzed at population level, revealed two major clusters, where all the populations of Upper Assam districts were in the same cluster with the original stock (control population) in a study conducted for the identification of genetic diversity among mutant lemon and mandarin varieties using different molecular markers (Akhtar *et al.*, 2024).

This study inferred that, among the 11 primers used for genetic diversity assessment in Coorg mandarin clones, only CMS4 and TAA33 were identified as highly informative and other primers were either moderately or less informative. Genetic distance values ranged from 0.00 to 0.90, reflecting considerable variation among accessions. Very close genetic relationships (0.00) were observed between accessions such as Kottageri and Kattemadu; also Sulugod and Kuttandi, which suggests potential clonal origin or recent divergence. The highest genetic distance (0.90) was recorded between Kanoor and Srimangala, which indicates significant genetic divergence. A dendrogram generated using Nei's genetic distance matrix and the UPGMA clustering method revealed several distinct clusters. One well-defined cluster consisted of Kanoor and Srimangala, suggesting a unique genetic identity potentially due to geographic isolation or specific ecological adaptation. Another major cluster included Boikeri, Perur, Sulugod and Kuttandi, indicates low genetic divergence and a likely shared regional gene pool, possibly due to similar ecological conditions and limited dispersal barriers. Overall, it can be said that, more number of SSR primers has to be employed to further analyze the genetic diversity present in Coorg mandarin.

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Genome-Wide Identification and Annotation of RNAi Machinery Genes in the Green Lacewing predator, *Chrysoperla zastrowi sillemi* (Neuroptera: Chrysopidae)

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Abstract

The green lacewing, *Chrysoperla zastrowi sillemi* (Esben-Petersen) (Neuroptera: Chrysopidae) commonly known as aphid lion, is an effective predator with significant potential in the biological control of insect pests. The RNA interference (RNAi) is a natural regulatory gene silencing mechanism present in eukaryotes. This technology relies on the introduction of double-stranded RNA (dsRNA) which is processed by the RNA interference machinery to bind and degrade complementary messenger RNA transcript of target genes, leading to their knockdown. dsRNA-based pesticides, while holding promise for specific insect control, raise safety concerns for natural enemies and non-target organisms. Since no published literature is available for the safety of pest specific dsRNA on *C. zastrowi sillemi*, the present study mined the core machinery genes facilitating the RNAi pathway from its genomic datasets (Gen Bank accession GCA_040669965.1) and identified these genes through various bioinformatics tools like tBLASTn and hmm search. RNAi core machinery, auxiliary factors regulating RISC, machinery genes involved in dsRNA transport, antiviral defense and dsRNA degrading nucleases were annotated and submitted to the NCBI database.

Keywords : *Chrysoperla zastrowi sillemi*, RNA interference, Machinery, Annotation

The common green lacewing, *Chrysoperla zastrowi sillemi* (Esben-Petersen) (Neuroptera: Chrysopidae) is an efficient biological control agent of insect and mite pests in various ecosystems (Nair *et al*., 2020). They are promising candidates of pest management worldwide owing to their wide prey range and geographical distribution, insecticide resistance, voracious larval feeding capability as well as ensured commercial availability. The green lacewing grubs are known to feed voraciously on aphids, mealybugs, coccids, thrips, psyllids, eggs and larvae of many lepidopteran pests and mites occurring on various crops (Pathak *et al*., 2022). The adult green lacewings feed solely on nectar, pollen and plant secretions containing sugar (Nair *et al*., 2020). The use of pesticides has led to various problems, including the development of resistance and resurgence of pests and residual toxic effects on humans, animals, insect parasites and predators (Ranadev *et al*., 2023). RNAi technology is a promising candidate next generation biopesticide, which can offer a sustainable and environment friendly pest management solution (Flynt, 2021).

RNA interference (RNAi) is an evolutionary conserved and ubiquitous gene-regulatory mechanism that exploits small double stranded RNA molecules to silence gene expression in a wide variety of eukaryotic organisms (Zhu and Palli, 2020). Silencing or knockdown of the target gene is elicited when a dsRNA targets a cognate messenger RNA (mRNA). RNAi has many species-specific variations, for example in the origin of the dsRNAs and in the number of homologous RNA silencing proteins expressed (Arraes *et al*, 2021). For the complete understanding of the RNA silencing process, the components of the RNA silencing machinery need to be identified.

In insects, three RNAi pathways are known, triggered by different classes of small RNAs, (i) small interfering RNAs (siRNAs) which mediate the siRNA pathway, (ii) micro RNAs (miRNAs) which mediate the miRNA pathway and (iii) P-element induced wimpy test is (Piwi)-interacting RNAs (piRNAs) which mediate the piRNA pathway (Zhu and Palli, 2020).

In small interfering pathway, long dsRNAs of exogenous or endogenous origin are cleaved by arthropod specific Dicer-2 (*Dcr2*), an RNase III enzyme with the help of R2D2 or Loquacious (*Loqs*) (Fig. 1a). The siRNA duplexes produced this way are

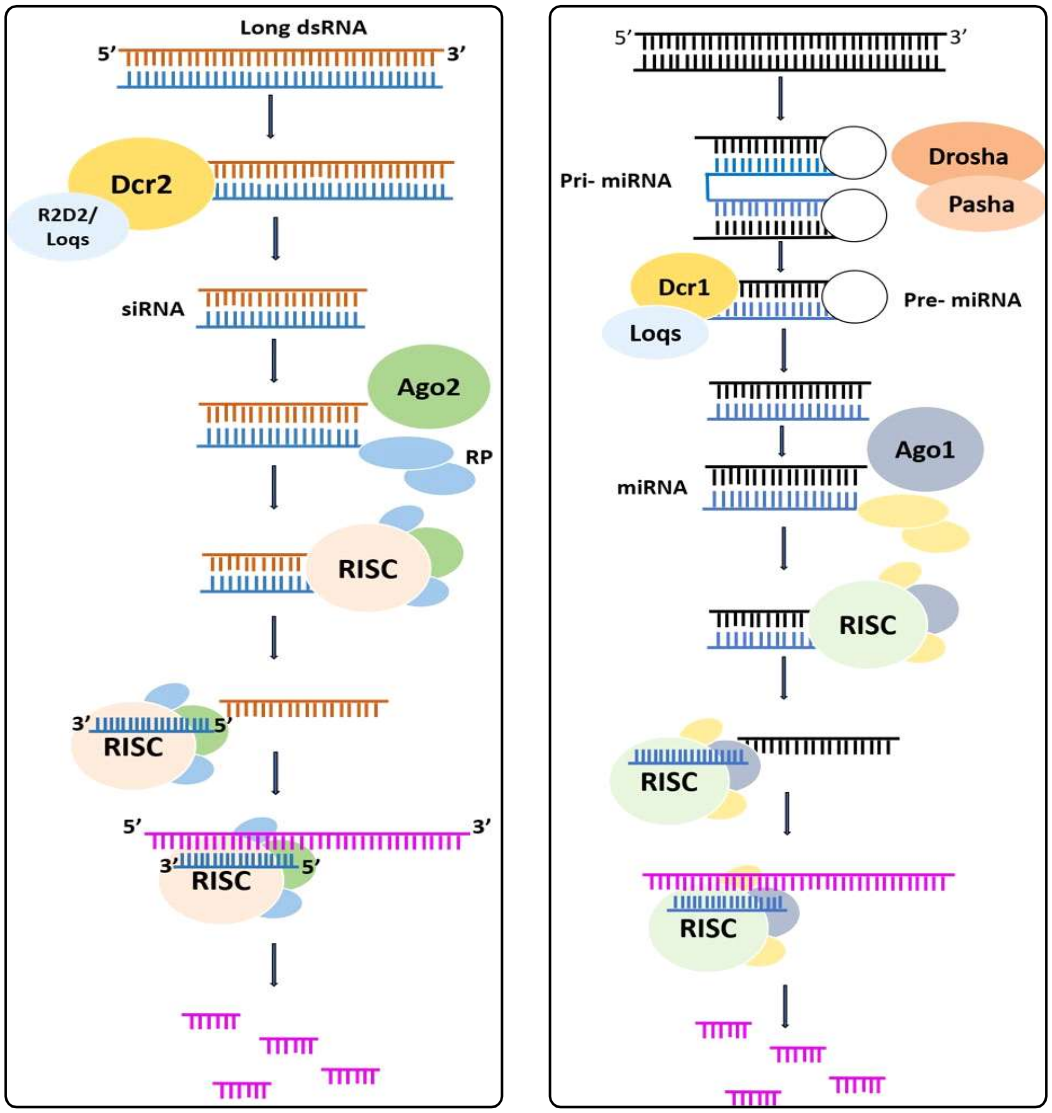

Fig. 1 : Schematic diagram of (a) siRNA pathway in insects (b) miRNA pathway in insects

typically of 19-21 bp length. Argonaute-2 (*Ago2*) and RISC associated proteins (RP) is assembled to form the RNA induced silencing complex (RISC), to which siRNA duplexes are loaded. The sense strand is then degraded and the antisense strand guides the cleavage of complementary target RNA by *Ago2* (Zhu and Palli, 2020). It is the siRNA pathway which is exploited when RNAi is experimentally induced. In miRNA pathway, dsRNA specific endoribonuclease, Drosha and its partner Pasha trim the primary miRNA which forms hairpin like pre-miRNA structure (Fig. 1b). Pre-miRNA is cleaved into miRNA duplexes by Dicer-1 (*Dcr1*). Argonaute-1 (*Ago1*) along with RISC associated proteins (RP) is assembled into RISC and mature miRNA duplexes are loaded and the complementary target mRNA is degraded by the *Ago1* enzyme. piRNA pathway is a dicer independent pathway of RNAi which provides defense against transposable elements. Piwi family proteins, Argonaute-3 (*Ago3*) and Aubergine (*Aub*) are involved in this pathway.

Interest in the use of RNAi technology for insect pest management has increased the necessity of studying RNAi pathways in beneficial insects also. Genomic and transcriptomic analysis are used to identify key players of RNAi machinery genes in many insect groups (Koo and Palli, 2024). The rigorously designed dsRNAs for the management of pest insect species may not cause any lethal effects to natural enemies. However, there are many factors that influence the safety and efficacy of dsRNA molecules and the understanding on the core RNAi machinery genes in non-target organisms is essential to ascertain potential detrimental effects. Hence, this study was designed to mine all the RNAi related genes from the genome of the biocontrol agent, *Chrysoperla zastrowi sillemi* to understand any potential off target effects.

Material and Methods

In this study, genome mining was carried out to verify the presence of important RNAi related genes. A list of RNAi related genes was compiled, covering the following categories; RNAi core machinery, auxiliary factors regulating RISC, machinery genes which are involved in dsRNA transport, antiviral defense and dsRNA degrading nucleases (Taning *et al*., 2016). Amino acid sequences of homologues of these genes from the closest insect organism, *Chrysoperla carnea* and model organisms like *Drosophila melanogaster* and *Tribolium castaneum* were retrieved from NCBI Genbank and used as query sequences to mine all the RNAi related genes (Castellanos *et al*., 2022). The genome of *Chrysoperla zastrowi sillemi,* available on NCBI (GenBank accession GCA_040669965.1) was then subjected to BLAST searches against the NCBI Non-redundant (NR) database (https://www.ncbi.nlm.nih.gov/refseq/about/non redundant proteins/), InterPro (https://www.ebi.ac.uk/interpro/) and Pfam (http://pfam.xfam.org/), setting the homology threshold to 10^{-5}. Hits obtained from this were subsequently used in tBLASTn (https://blast.ncbi. nlm.nih.gov/Blast.cgi) searches to verify their identity and to annotate them (Taning *et al*., 2016). The identification of the RNAi machinery genes was done based on maximum score and query coverage in the BLAST results (Pandit *et al*., 2023). The annotated sequences of RNAi machinery genes were submitted to NCBI GenBank. The tBLASTx results were tabulated with necessary details like top hit organism, its accession ID, E value, per cent identity and NCBI accession ID of the RNAi machinery genes of *C. zastrowi sillemi*.

Results and Discussion

The core RNAi machinery genes in an organism is constituted by Dicer enzymes, Argonaute endonucleases and dsRNA binding proteins (Swevers *et al*., 2013). The presence of machinery genes of all three different RNAi pathways were identified from the genome of *C. zastrowi sillemi*. The siRNA pathway genes; Dicer-2 and Argonaute-2 are present in the genome (Table 1). Dicer-2 is involved in the processing of long dsRNAs to siRNAs. Argonaute-2 forms the catalytic subunit of RISC and tightly binds to single stranded siRNA and cleaves target RNA in target RNA or siRNA hybrids. However, R2D2, the dsRNA cofactor of Dicer-2 was not identified from the genome.

TABLE 1

Core machinery genes of siRNA pathway identified from the genome of *C. zastrowi sillemi*

siRNA core genes	Organism	Accession	Query cover	E value	Identity (%)	NCBI accession *C. zastrowi sillemii*
Dicer-2	*Chrysoperla carnea*	XM_044882977.1	100%	0.0	97.00%	V739941
Argonaute-2	*Chrysoperla carnea*	XM_044881195.1	100%	0.0	100.00%	V739913

The miRNA pathway constituted by nuclear RNAse III enzyme Drosha and its partner, Pasha which is involved in processing of miRNA precursors and cytoplasmic RNAse III enzyme, Dicer-1 along with its cofactor Loquacious, which catalyse the maturation of small RNAs are present in the *C. zastrowi sillemi* genome (Table 2). Argonaute proteins of the piwi class take part in the piRNA pathway which provides defense against transposable elements. Piwi like protein, Siwi and Argonaute-3, catalytic units of RISC of the piRNA pathway are encoded by the genome of *C. zastrowi sillemi* (Table 3).

Similar to our investigation, Castallenos *et al.* (2022) screened the transcriptome of parasitoid wasp, *Telenomus podisi* for the presence of most important genes related to RNAi machinery. All the genes representing the core proteins of the three RNAi pathways found in insects siRNA, miRNA and piRNA were identified, except R2D2 from the transcriptome of *T. podisi*. In the absence of R2D2, Dicer-2 is still capable of efficiently processing long dsRNA into siRNAs using the machinery gene loquacious, that has function comparable to R2D2 (Reddy *et al*., 2025). The presence of RNAi machinery genes of three main

TABLE 2

Core machinery genes of miRNA pathway identified from the genome of *C. zastrowi sillemi*

miRNA core genes	Organism	Accession	Query cover	E value	Identity (%)	NCBI accession *C. zastrowi sillemii*
Dicer-1	*Chrysoperla carnea*	XM_044885558.1	100%	0.0	90.02%	V739914
Drosha	*Chrysoperla carnea*	XM_044885390.1	93%	0.0	94.27%	V739916
Pasha	*Chrysoperla carnea*	XM_044885645.1	100%	0.0	82.68%	V739915
Loquacious	*Chrysoperla carnea*	XM_044878676.1	100%	0.0	93.99%	V739917

TABLE 3

Core machinery genes of piRNA pathway identified from the genome of *C. zastrowi sillemi*

piRNA core genes	Organism	Accession	Query cover	E value	Identity (%)	NCBI accession *C. zastrowi sillemii*
Siwi	*Chrysoperla carnea*	XM_044884311.1	92%	0.0	96.06%	V739919
Argonaute-3	*Chrysoperla carnea*	XM_044882093.1	100%	0.0	90.95%	V739918

TABLE 4

Auxiliary factors related to RNAi identified from the genome of *C. zastrowi sillemi*

Auxiliary factors (RISC)	Organism	Accession	Query cover	E value	Identity (%)	NCBI accession *C. zastrowi sillemii*
Translin	*Chrysoperla carnea*	XM_044882727.1	100%	1e-165	93.55%	V739920
Trax-B	*Chrysoperla carnea*	XM_044874101.1	100%	0.0	100.00%	V739921
Gawky	*Chrysoperla carnea*	XM_044878328.1	100%	0.0	77.48%	V739922
FXMR	*Chrysoperla carnea*	XM_044880460.1	100%	0.0	81.35%	V739924
Belle	*Chrysoperla carnea*	XM_044870674.1	100%	0.0	76.63%	V739926
p68 RNA helicase	*Chrysoperla carnea*	XM_044880450.1	83%	0.0	95.50%	V739923
Armitage	*Chrysoperla carnea*	XM_044879498.1	100%	0.0	96.67%	V739925
Staufen	*Chrysoperla carnea*	XM_044885430.1	98%	0.0	76.79%	V739927
Maelstrom	*Chrysoperla carnea*	XM_044872891.1	100%	0.0	96.37%	V739928
PRMT5	*Chrysoperla carnea*	XM_044875904.1	100%	0.0	99.67%	V739929
Clp-1	*Chrysoperla carnea*	XM_044872687.1	100%	0.0	85.08%	V739930

pathways was similarly studied in various insects; *Spodoptera litura* by Gong *et al.* (2015), *Agrilus planipennis* by Zhao *et al.* (2015), *Diabrotica v irgifera virgifera*, *Spodoptera frugiperda* and *Nezara viridula* by Davis-Vogel *et al.* (2018) and *Anastrepha fraterculus* by Dias *et al.* (2019).

Auxillary factors are associated with the or regulate the activity of RISC complexes. Genes like Translin, Trax-B, Gawky, Belle, FXMR, p68 RNA helicase, Staufen, Armitage, Maelstrom, PRMT-5 and Clp-1 were mined from the genome of *C. zastrowi sillemi* (Table 4). SID1, FBX011, Exportin-1, Exportin-5 genes which are associated with the dsRNA transport were identified (Table 5). Homologues of SID1 proteins are present in insects which is thought to be involved in transport of dsRNA across the plasma membrane (Swevers *et al*., 2013). The RNAi genes involved in the antiviral defense are Arsenite resistance protein, *Ars2* and Egghead (Table 6). Nibbler and RNA exonucleases (*Rex5, Rex1* and *Rex4*) are dsRNA degrading enzymes found in *C. zastrowi sillemi* (Table 7). An overview of the RNA interference related genes identified from the genome with brief description of their functions is presented in Table 8.

TABLE 5

RNAi machinery genes involved in the transport of dsRNA identified from the genome of *C. zastrowi sillemi*

dsRNA transport	Organism	Accession	Query cover	E value	Identity (%)	NCBI accession *C. zastrowi sillemii*
SID1	*Chrysoperla carnea*	XM_044882095.1	100%	0.0	96.99%	V739937
FBX011	*Chrysoperla carnea*	XM_044872004.1	100%	0.0	88.81%	V739940
Exportin-1	*Chrysoperla carnea*	XM_044876395.1	100%	0.0	97.35%	V739939
Exportin-5	*Chrysoperla carnea*	XM_044880269.1	100%	0.0	97.18%	V739938

TABLE 6

RNAi machinery genes involved in antiviral defense identified from the genome of *C. zastrowi sillemi*

Antiviral RNAi	Organism	Accession	Query cover	E value	Identity (%)	NCBI accession *C. zastrowi sillemii*
Ars2	*Chrysoperla carnea*	XM_044875637.1	87%	0.0	87.29%	V739931
Egghead	*Chrysoperla carnea*	XM_044879903.1	93%	0.0	99.63%	V739932

TABLE 7

dsRNAses identified from the genome of *C. zastrowi sillemi*

Nucleases	Organism	Accession	Query cover	E value	Identity (%)	NCBI accession *C. zastrowi sillemii*
Nibbler	*Chrysoperla carnea*	XM_044875780.1	100%	0.0	95.31%	V739933
Rex 5	*Chrysoperla carnea*	XM_044881656.1	100%	0.0	93.59%	V739934
Rex1	*Chrysoperla carnea*	XM_044885394.1	76%	0.0	97.38%	V739935
Rex4	*Chrysoperla carnea*	XM_044885686.1	100%	0.0	93.19%	V739936

TABLE 8

Overview of RNA interference factors mined from the genome of *C. zastrowi sillemi*, with a brief description of their function

RNAi related genes	Function
Dicer-2	dsRNA specific RNase-III-type endonucleases which catalyse the maturation of small RNAs
Argonaute-2	Component of RISC, tightly binds to single stranded siRNA and cleaves target RNA in target RNA/siRNA hybrids
Dicer-1	dsRNA specific RNase-III-type endonucleases which catalyse the maturation of small RNAs
Drosha	dsRNA specific RNase-III-type endonucleases involved in processing of miRNA precursors
Pasha	dsRNA-binding, essential co-factor of Drosha
Loquacious	dsRNA-binding, essential co-factor of Dicer-1, paralog of R2D2
Siwi	Argonauteof PIWI subfamily which is a catalytic subunit of RISC
Argonaute-3	Argonaute of PIWI subfamily which is a catalytic subunit of RISC
Translin	RNA-binding protein and endonuclease complex, triggers miRNA and siRNA pathway
Trax-B	RNA-binding protein and endonuclease complex, triggers miRNA and siRNA pathway
Gawky	RNAi effector, interacts with Argonaute proteins
FXMR	Fragile-X-related protein, component of the RISC complex of S2 cells
Belle	DEAD-box RNA helicase which interacts with small RNAs and RISC
p68 RNA helicase	DEAD-box RNA helicase, involved in RNAi

Continued....

TABLE 8 Continued....

RNAi related genes	Function
Armitage	ATP-dependent helicase required for RISC maturation, nuclease involved in piRNA biogenesis, miRNP assembly
Staufen	Tubulin-binding and dsRNA-binding domain involved in the transport of mRNA
Maelstrom with	piRNA pathway domain weak similarity to HMG box, Mael domain with weak homology the DnaQ-H 32 -to-52 exonuclease
PRMT5	Protein methyltransferase that methylates Piwi proteins at conserved Arg-residues
Clp-1	RNA kinase involved in phosphorylation of thesiRNAs
SID1	Homolog of the putative dsRNA transporter
FBX011	Involved in the degradation of proteins involved in dsRNA uptake (endocytosis)
Exportin-5	Export pre-microRNA (also known as pre-miRNA) out of the nucleus and into the cytoplasm
Exportin-1	Nuclear export of various RNA species
Ars2	Regulator of siRNA- and miRNA-mediated silencing, suppressor of RNA virus infection
Egghead	Uptake of dsRNA, innate immunity against RNA virus
MUT-7/Nibbler	Processing of 32 ends of miRNAs (Drosophila)
Rex 5/ Rex 4/ Rex 1	Single-strand-specific small RNA degrading nucleases; degradation of mature miRNAs

Similar to this investigation, identification and analysis of RNAi related genes in Asian citrus psyllid, *Diaphorina citri*, unveiled the presence of various auxillary factors, factors related to dsRNA uptake, antiviral RNAi and nucleases (Taning *et al*., 2016). Tian *et al.* (2019) comprehensively identified 33 potential genes involved in the RNAi pathway in *Bemisiata baci* genome, putatively associated with biosynthesis, transmission, regulation and others, similar to our findings.

Analysis of various insect genomes show variations in the number of core RNAi pathway genes between different major insect groups (Dowling *et al*., 2016). All sequences representing the core RNAi machinery genes of *C.zastrowi sillemi* were identified in the genome, except for R2D2, cofactor of Dicer-2 in siRNA pathway and Argonaute-1 of miRNA pathway. The absence of these genes could be attributed to low transcript abundance or gene deletion (Reddy *et al*., 2025). The RNAi machinery genes of *C. zastrowi sillemi* share more identity with its sibling species *C. carnea* as revealed by the BLAST analysis. Importantly, the presence of Sid-1 was confirmed in *C.zastrowi sillemi*, which is absent in the model organism, *Drosophila melanogaster* (Order Diptera). Duplication of core RNAi machinery genes as in case of model organisms like *Tribolium castaneum* (Order Coleoptera) and *Drosophila melanogaster* (Order Diptera) has not been observed in the genome of *C. zastrowi sillemi.*

Variability in RNAi silencing efficacy among insects may be partially explained by differences in the diversity and copy number of RNAi pathway genes present across lineages. Although RNAi has been extensively studied in several insect orders such as Diptera, Lepidoptera, Hemiptera and Coleoptera, no consolidated literature currently exists on the RNAi machinery genes in Neuroptera. Despite being a well exploited natural enemy, the genomic makeup of *Chrysoperla zastrowi sillemi* has not been analyzed and only limited sequence information is available. In this study, we confirmed the presence of 29 RNAi machinery genes in the genome of *C. zastrowi sillemi* through in silico analysis. Identification and analysis of core genes for the RNAi pathway encoded by the genome suggests the presence of functional RNAi pathways in this beneficial insect. This investigation provides the first detailed account of RNAi machinery

genes in the insect order Neuroptera. These findings provide valuable ground information for the future research on the exploitation of RNAi in *C. zastrowi sillemi.* It also lays the foundation for RNAi-based functional genomics research and safety assessment to determine the ecological safety of dsRNA constructs on this beneficial insect.

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A Scale to Analyze the Perception of Agricultural Input Dealers on Diploma in Agricultural Extension Services (DAESI) Programme

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Abstract

An attempt was made in the present study to develop and standardize scale to analyze the perception of Agricultural Input Dealers on Diploma in Agricultural Extension Services (DAESI) programme. The developed perception scale was found to be highly reliable (0.870) and valid (0.940). The perception scale consists of 46 statements classified in to six components. The developed perception scale was administered to 50 DAESI input dealers in KVK, Bengaluru (Rural) district of Karnataka state during 2024-25. The results revealed that majority of the DAESI input dealers (96.0%) had good to better perception towards DAESI programme, while 4.00 per cent of the input dealers had poor perception towards DAESI programme. The variables like decision-making and risk-taking ability had strong positive correlation with 1 per cent level of significance. This demonstrates that input dealers who are more capable of taking risks, making creative decisions and using new strategies typically have a more favourable opinion on the DAESI programme. Other traits like leadership, motivation, scientific orientation and social participation were also positively related at 5 per cent level. Thus, entrepreneurial and progressive dealers with participatory attitudes tend to view DAESI programme more favourably. All the 18 independent variables have contributed to the tune of 58.10 per cent ($R^2 = 0.581$) in developing better perception of DAESI input dealers towards DAESI programme.

Keywords : DAESI, Perception, Agri input dealers, Scale development

India's primary source of income, particularly in rural areas, comes from agriculture and related businesses. Food security, the creation of employment in rural areas, and the preservation of the environment all rely on agriculture, which also contributes significantly to the nation's GDP. It is crucial to use sustainable agricultural methods such as soil conservation, effective natural resource management, and biodiversity preservation. Revolutions like the Green, White, Yellow and Blue Revolutions have had a significant impact on India's agriculture. Majority of people in rural areas depend upon farming as their primary source of income, so enhancing this industry contributes to a reduction in poverty and hunger. Development in agriculture can help to create a more equitable society and is frequently more effective at lowering poverty than growth in other areas. The government makes significant investments in agricultural research, education and extension services. These contribute to higher farm income and production. Public extension workers are limited and frequently

preoccupied with other duties, but extension services serve as a bridge between farmers and research. Consequently, farmers might not always receive appropriate guidance on modern farming methods.

At present in India approximately 2.82 lakh input dealers provide seeds, fertilizer and other agricultural inputs to the farmers. Above all they educate the farmers on recent agricultural technologies. Since these agricultural input dealers are locally available the farmers rely upon them to a greater extent for information on agriculture. With this background, the government of India has been educating input dealers. To teach agricultural input dealers about current farming methods, MANAGE and Agricultural Universities have developed specialized training programmes. The Diploma in Agricultural Extension Services for Input Dealers (DAESI), which was launched by MANAGE in 2003, is one such programme. It prepares input dealers to serve as para-extension professionals and offer guidance according to local agricultural needs. DAESI is a Central Sector Plan scheme and is being implemented throughout the India. Its aim is to turn input dealers into better service providers to the farmers. The course mainly covers crop production techniques, laws about farm inputs and proper input use. In Karnataka since 2016, dealers are trained by Nodal Training Institutes under SAMETI (North) and SAMETI (South). The present study was undertaken with the following specific objectives;

1. To develop a scale to analyse the perception of DAESI input dealers towards DAESI programme
2. To find out the relationship between profile characteristics of DAESI input dealers with their perception towards DAESI programme.

METHODOLOGY

The present study was carried out during 2024 for developing and standardizing a scale to analyse the perception of Agricultural Input Dealers on Diploma in Agricultural Extension Services (DAESI) programme. The developed scale was used to analyze the perception of DAESI input dealers on DAESI programme in Bengaluru (Rural) district of Karnataka state. Fifty DAESI input dealers trained by KVK, Bengaluru (Rural) were personally interviewed using pretested interview schedule for the purpose. The results were expressed in terms of frequency and percentages. The data collected were scored, tabulated and analyzed using frequency, mean, percentage, correlation test and multiple regression analysis.

RESULTS AND DISCUSSION

Development of Scale to Analyze the Perception of DAESI Input Dealers towards DAESI Programme

Perception of DAESI input dealers towards DAESI programme is operationally defined in the present study 'as the extent of mental awareness of DAESI input dealers and farmers towards DAESI programme. The method of summated rating scale suggested by Likert (1932) [2] and Edwards (1969) [1] were followed in the development of the perception scale, following six stages *viz.,* (1) identification of components, (2) collection and editing of perception statements, (3) relevancy test, (4) item analysis, (5) reliability and (6) validity.

Identification of Components

Six components related to perception of DAESI input dealers towards DAESI programme were identified based on review of literature and discussion with extensionists. The identified six components includes perception towards Perception on course procedure, methodology and content, Perception on classroom teaching, Perception on practical classes, Perception on record maintenance & assignments, Perception on business enterprise and Perception as para extension workers.

Collection and Editing of Perception Statements

The first step in the construction of perception scale was to collect statements pertaining to the perception

of DAESI input dealers towards DAESI programme. A tentative list of 83 statements pertaining to the perception of DAESI input dealers towards DAESI programme were collected through extensive review of literature and by consulting extensionists. These 83 statements were edited as per the 14 criteria enunciated by Edwards (1969. As a consequence, 12 statements were eliminated. The remaining 71 perception statements were included for the study.

Relevancy Test : Seventy-one statements were sent to 96 experts/judges in the field of social sciences working in State Agricultural Universities, ICAR Institutes and Development Departments to critically evaluate the relevancy of each statement *viz.*, Most Relevant (MR), Relevant (R), Somewhat Relevant (SWR), Less Relevant (LR) and Not Relevant (NR) with the score of 5, 4, 3, 2 and 1, respectively. The experts/judges were also requested to make necessary modifications and additions or deletion of perception statements, if they desired to. A total of 68 judges/experts returned the questionnaires duly completed and the perception statements were considered for further processing. From the data gathered, 'relevancy percentage (RP)' and 'mean relevancy score (MRS)' were worked out for all the 71 statements. Using these criteria, individual perception statements were screened for relevancies using the following formulae.

i) Relevancy Percentage (RP) : It was obtained by using the following formulae

$$R.P. = \frac{\text{MR} \times 5 + \text{R} \times 4 + \text{SWR} \times 3 + \text{LR} \times 2 + \text{NR} \times 1}{Maximum\ possible\ score} \times 100$$

ii) Mean Relevancy Score (MRS): It was worked out using the following formula

$$M.R.S = \frac{\text{MR} \times 5 + \text{R} \times 4 + \text{SWR} \times 3 + \text{LR} \times 2 + \text{NR} \times 1}{Number\ of\ judges/\ experts\ responded}$$

Accordingly, statements having 'relevancy percentage' of 80 per cent and above and mean relevancy score of 4.00 and above were considered for final selection. Accordingly, 56 perception statements were retained after relevancy test and these statements were suitably modified and written as per the comments of the judges wherever applicable.

Item analysis : Fifty-six perception statements were subjected to item analysis to delineate the items based on the extent to which they can differentiate the respondent having better perception from the respondent with poor perception regarding DAESI programme.

A sample of 32 DAESI input dealers in Chikmagalur district of Karnataka state were selected for the study. The respondents were asked to indicate their degree of agreement or disagreement with each of the 56 perception statements on a five-point continuum ranging from 'strongly agree' to 'strongly disagree'. Based upon the total scores, the respondents were arranged in descending order. The top 25 per cent of the respondents with their total scores were considered as the high group and the bottom 25 per cent as the low group. These two groups provided criterion groups in terms of evaluating the individual statements. Thus, out of 32 DAESI input dealers to whom the perception statements were administered for item analysis, eight DAESI input dealers with highest and eight DAESI input dealers with lowest perception scores were used as criterion groups to evaluate individual items. The critical ratio, that is, the 't' value which analyses the extent to which a given statement differentiates between the better and poor groups of respondents for each statement, was calculated by using the following formula :

$$t = \frac{\overline{X}_H - \overline{X}_L}{\sqrt{\frac{\sum X_H^2 - \frac{(\sum X_H)^2}{n} \times \sum X_L^2 - \frac{(\sum X_L)^2}{n}}{n(n-1)}}}$$

Where,

XH = The mean score on given statement of the high group

XL = The mean score on given statement of the low group

∑X2H = Sum of squares of the individual score on a given statement for high group

∑X2L = Sum of squares of the individual score on a given statement for low group

n = Number of respondents in each group

∑ = Summation

t = The extent to which a given statement differentiates between the high and low groups.

After computing the 't' value for all the 56 items, forty-six perception statements with 't' value equal to or greater than 1.692 (which were significant for at least 5% level) were finally selected and included in the final perception scale.

Reliability : Reliability refers to precision of the scale constructed for any purpose. A reliability test will be reliable when it gives the same repeated result under the same conditions. In any social science research, a newly constructed scale has to be tested for its reliability before it is used. The split-half method was employed to test the reliability of the perception scale. The value of correlation coefficient was 0.870 and this was further corrected by using Spearman Brown formula to obtain the reliability coefficient of the whole set. The 'r' value of the scale was 0.770, which was significant at one per cent level indicating the high reliability of the scale. It was concluded that the perception scale constructed was reliable.

a) Half test reliability formula

$$r\ 1/2 \quad \frac{N(\sum XY) - (\sum X)(\sum Y)}{\sqrt{(N\sum X^2 - (\sum X)^2)\,(N\sum Y^2 - (\sum Y)^2)}}$$

Where,

∑X = Sum of the scores of the odd number items

∑Y = Sum of the scores of the even number items

∑X2 = Sum of the squares of the odd number items

∑Y2 = Sum of the squares of the even number items

b) Whole test reliability formula

$$r_{1/1} = \frac{2_{r1/2}}{1 + r_{1/2}}$$

Where,

r1/2= Half test reliability

Validity : It refers to how well a scale analyses what it is purported to measure. The data was subjected to statistical validity, which was found to be 0.940 for scale which is greater than the standard requirement of 0.756 (Validity = √r11). Hence, the validity coefficient was also found to be appropriate and suitable for the tool developed. Thus, the developed scale to analyze the perception of DAESI input dealers towards DAESI programme was feasible and appropriate.

Administration of Perception Scale and Method of Scoring

The final scale consists of 46 statements for determining the perception of DAESI input dealers towards DAESI programme. The responses were collected on a five-point continuum, namely, strongly agree, agree, undecided, disagree and strongly disagree with assigned score of 5, 4, 3, 2 and 1, respectively. The perception score of a respondent was calculated by adding up the scores obtained by him/her on all the 46 statements. The perception score of this scale ranges from a minimum of 46 score to a maximum of 230 score. Based on the mean and half standard deviation the respondents were categorized into three perception categories, *viz.*, poor, good and better.

Statement wise distribution of DAESI input dealers towards Perception of DAESI programme

The developed scale was administered to 50 DAESI input dealers trained by KVK, Bengaluru (Rural) district of Karnataka state during 2024-25. The results in Table 2 showed the analysis of the DAESI input dealers across various components provides insight

TABLE 1

Scale to analyse the perception of DAESI input dealers towards DAESI programme

Perception Statements	Strongly agree	Agree	Undecided	Disagree	Strongly disagree
Perception on course procedure, methodology and content					
The classroom hours of 10 AM to 5 PM is convenient					
Study materials are provided in the local languages					
Exams are carried out in a highly impartial and strict manner.					
The duration of the DAESI programme is appropriate					
Providing the uniform to input dealers will give them an identity and also visibility to the programme					
Diploma certificate is awarded in time to input dealers					
80 % compulsory attendance in the classroom and practical class is necessary					
Perception on classroom teaching					
The content covered in the classrooms are relevant to the topics					
There is no utilization of multimedia instructional materials in the classroom.					
The study material provided by the institution is on par with the topics covered in the classrooms					
The resource persons are having sound knowledge on the topics to be covered as well as good communication skills					
At least 30 % of the content of the syllabus is location-specific					
Equal weightage is given to theory, practical and assignments					
The use of different teaching aids will hold the attention of input dealers and also facilitate easy understanding					
Perception on practical classes					
The number of field visits organized to different institutions and agro-industries is sufficient.					
Laboratories are well equipped and sufficient for all the participants					
Live specimens will not be used for showing the symptoms and severity of insect attacks and crop diseases.					
Participants get to see demonstration plots showcasing various crops and farming techniques, which help in understanding practical applications.					
Hands-on training on the correct methods of fertilizer application will be provided to ensure optimal crop nutrition and minimize waste					
Practical field visits and hands-on training provide dealers with real-world experience, enhancing their ability to offer practical solutions in extension services					
Perception on record maintenance and assignments					
Participants are required to complete regular assignments to reinforce learning and demonstrate understanding of course material					
Attendance register is maintained from the beginning day till the end of the programme to ensure regular participation of input dealers					
Regular feedback on assignments helps participants improve their record-keeping practices					

Continued....

TABLE 1 Continued....

Perception Statements	Strongly agree	Agree	Undecided	Disagree	Strongly disagree
Each input dealer shall maintain a 'problem-solution record' containing queries of the farmers during their visits to input dealers and the advice rendered by them					
Participants prepare and deliver presentations on various agricultural topics covered in the programme					
Assignments include creating extension leaflets or brochures on best farming practices					
Dealers who receive training in inventory management and record keeping are not able to maximize stock levels, cut waste and enhance cash flow.					
Perception on business enterprise					
Input dealers are supplying inputs including fertilizers, plant protection chemicals and seeds in prescribed quantities rather than in excess due to knowledge obtained through the DAESI programme					
Dealers gain up-to-date knowledge about modern agricultural practices, which can improve the quality of advice they provide to farmers, leading to increased customer trust and loyalty					
After knowing the importance of promotional activities, dealers are now engaged in promoting the good agricultural practices which intern benefits the farmer.					
The ability to recommend appropriate inputs based on scientific knowledge can lead to decreased sales					
Dealers may diversify their product offerings based on the latest agricultural trends and technologies learned during the programme, attracting a broader customer base					
The programme can provide insights into market trends and customer preferences, allowing dealers to develop more effective marketing strategies and promotional activities					
Improved professional skills and networking opportunities do not assist dealers in establishing stronger relationships with all stakeholders.					
Improved understanding of agricultural risks and mitigation strategies helps dealers better manage business risks and uncertainties					
Training in record maintenance and data analysis enables dealers to make informed, data-driven business decisions					
The programme fosters leadership skills, empowering dealers to take on more significant roles and responsibilities within their business and the agricultural community					
Perception as Para-extension workers					
By imparting the necessary expertise, input dealers have the potential to become para-extension experts who may professionalize extension services and bring about a paradigm shift in Indian agriculture					
To offer extension services to the farming community, separate extension corners have been established inside the presently operating unit.					
Before the DAESI programme, farmers were not encouraged to bring samples in order to get a solution, but dealers are now requesting that they do.					
The programme introduces digital tools and technologies (DVD, CD and T.V. etc.,) that dealers can use in their shops to enhance their extension services and reach a wider audience					
The programme includes training on interacting with farmers, understanding their needs, and building trust, which are essential qualities of a para-extension worker					

Continued....

TABLE 1 Continued....

Perception Statements	Strongly agree	Agree	Undecided	Disagree	Strongly disagree
A client-cantered approach to extension work that prioritizes the requirements and preferences of specific farmers will not be encouraged by the programme.					
Input dealers as a para extension worker helps in selecting beneficiaries to the different agricultural programme					
Input dealers coordinate with agricultural and allied departments and agricultural universities in engaging extension educational activities					
These para extension workers help in planning and implementation of various agricultural programme					

into their perception on DAESI programme. It is observed that among the seven perception statements with respect to the perception on course procedure, methodology and content, the statements 'The classroom hours of 10 AM to 5 PM is convenient' and 'Exams are carried out in a highly impartial and strict manner' obtained a mean perception score of 5.00 and was ranked first, while the statements 'Diploma certificate is awarded in time to input dealers' and '80 per cent compulsory attendance in the classroom and practical class is necessary' received a mean perception score of 4.80 and were ranked third.

The statements 'The duration of the DAESI programme is appropriate' and 'Study materials are provided in the local languages' obtained a mean perception score of 4.60 and was ranked fifth by the DAESI input dealers, whereas the statement 'Providing the uniform to input dealers will give them an identity and also visibility to the programme was ranked seventh with mean perception score 3.80. Among the seven perception statements with respect to the perception on classroom teaching of DAESI programme the statements 'The content covered in the classrooms are relevant to the topics' and 'There is no utilization of multimedia instructional materials in the classroom' obtained a mean perception score of 5.00 and was ranked first. The statements, 'The use of different teaching aids will hold the attention of input dealers and also facilitate easy understanding' and 'Equal weightage is given to theory, practical and assignments' received a mean perception score of 4.80 and were ranked third. The remaining statements 'The resource persons are having sound knowledge on the topics to be covered as well as good communication skills', 'At least 30 per cent of the content of the syllabus is location-specific' and 'The facilitator ensures that the selected venue has the necessary audio-visual aids for teaching that will hold the attention of input dealers and also facilitate easy understanding' were ranked fifth (4.60), sixth (4.40) and seventh (4.20) respectively.

In the component 'perception on practical classes', the statement 'Participants get to see demonstration plots showcasing various crops and farming techniques, which help in understanding practical applications' was ranked first (5.00) by KVK trained input dealers, highlighting their belief as demonstration plots are highly effective in enhancing practical understanding and are the most valued component of the DAESI training by input dealers. In the fourth component 'perception on record maintenance and assignments', the statement 'Participants are required to complete regular assignments to reinforce learning and demonstrate understanding of course material' was ranked first, which indicates that assignments helped dealers revise, understand better and stay engaged.

Among ten statements in fifth component 'perception on business enterprise', the statement 'Input dealers are supplying inputs including fertilizers, plant protection chemicals and seeds in prescribed quantities rather than in excess due to knowledge obtained through the DAESI programme' was ranked

first, highlights the input dealers enhanced knowledge, enabling them to supply farm inputs in recommended quantities and thereby support sustainable farming practices. In the last component *i.e.,* 'perception as para-extension workers', the statement, 'By imparting the necessary expertise, input dealers have the potential to become para-extension experts who may professionalize extension services and bring about a paradigm shift in Indian agriculture' was ranked first in KVK (score: 5.00), illuminates that DAESI applicants strappingly recognized the programme's role in empowering input dealers with proficiency to act as para-extension experts. The impact of the DAESI programme can be strengthened by enriching the curriculum with more location-specific content, digital learning materials and interactive teaching methods. Increasing the number of practical demonstrations and field visits while improving business and advisory skill training will further enhance participants' competence. Continuous feedback, updated resource-person training and periodic programme refinement will help sustain quality and relevance.

TABLE 2

Statement wise distribution of DAESI input dealers towards Perception of DAESI programme

(n=50)

Statements	Mean perception score	Rank
Perception on course procedure, methodology and content		
The classroom hours of 10 AM to 5 PM is convenient	5.00	I
Exams are carried out in a highly impartial and strict manner.	5.00	I
Diploma certificate is awarded in time to input dealers	4.80	III
80 % compulsory attendance in the classroom and practical class is necessary	4.80	III
The duration of the DAESI programme is appropriate	4.60	V
Study materials are provided in the local languages	4.60	V
Providing the uniform to input dealers will givethem an identity and also visibility to the programme	3.80	VII
Perception on classroom teaching		
The content covered in the classrooms are relevant to the topics	5.00	I
There is no utilization of multimedia instructional materials in the classroom.	5.00	I
Equal weightage is given to theory, practical and assignments	4.80	II
The use of different teaching aids will hold the attention of input dealers and also facilitate easy understanding	4.80	II
The resource persons are having sound knowledge on the topics to be covered as well as good communication skills	4.60	V
At least 30 % of the content of the syllabus is location-specific	4.40	VI
The facilitator ensures that the selected venue has the necessary audio-visual aids for teaching that will hold the attention of input dealers and also facilitate easy understanding	4.20	VII
Perception on practical classes		
Participants get to see demonstration plots showcasing various crops and farming techniques, which help in understandingpractical applications.	5.00	I
Laboratories are well equipped and sufficient for all the participants	4.80	II

Continued....

TABLE 2 Continued....

Statements	Mean perception score	Rank
Hands-on training on the correct methods of fertilizer application will be provided to ensure optimal crop nutrition and minimize waste	4.60	III
Practical field visits and hands-on training provide dealers with real-world experience, enhancing their ability to offer practical solutions in extension services	4.60	III
The number of field visits organized to different institutions and agro-industries is sufficient.	4.40	V
Live specimens will not be used for showing the symptoms and severity of insect attacks and crop diseases.	4.20	VI
Perception on record maintenance and assignments		
Participants are required to complete regular assignments to reinforce learning and demonstrate understanding of course material	5.00	I
Attendance register is maintained from the beginning day till the end of the programme to ensure regular participation of input dealers	4.80	II
Regular feedback on assignments helps participants improve their record-keeping practices	4.60	III
Dealers who receive training in inventory management and record keeping are not able to maximize stock levels, cut waste, and enhance cash flow.	4.60	III
Each input dealer shall maintain a 'problem- solution record' containing queries of the farmers during their visits to input dealers and the advice rendered by them	4.40	V
Participants prepare and deliver presentations on various agricultural topics covered in the programme	4.40	V
Assignments include creating extension leaflets or brochures on best farming practices	4.40	V
Perception on business enterprise		
Input dealers are supplying inputs including fertilizers, plant protection chemicals and seeds in prescribed quantities rather than in excess due to knowledge obtained through the DAESI programme	5.00	I
The programme can provide insights into market trends and customer preferences, allowing dealers to develop more effective marketing strategies and promotional activities	5.00	I
Improved understanding of agricultural risks and mitigation strategies helps dealers better manage business risks and uncertainties	5.00	I
Dealers may diversify their product offerings based on the latest agricultural trends and technologies learned during the programme, attracting a broader customer base	4.80	IV
Dealers gain up-to-date knowledge about modern agricultural practices, which can improve the quality of advice they provide to farmers, leading to increased customer trust and loyalty	4.60	V
Training in record maintenance and data analysis enables dealers to make informed, data-driven business decisions	4.60	V
After knowing the importance of promotional activities, dealers are now engaged in promoting the good agricultural practices which intern benefits the farmer.	4.40	VII

Continued....

TABLE 2 Continued....

Statements	Mean perception score	Rank
The ability to recommend appropriate inputs based on scientific knowledge can lead to decreased sales	4.40	VII
The programme fosters leadership skills, empowering dealers to take on more significant roles and responsibilities within their business and the agricultural community	4.00	IX
Improved professional skills and networking opportunities do notassist dealers in stablishing stronger relationships with all stakeholders.	3.80	X
Perception as Para-extension workers		
By imparting the necessary expertise, input dealers have the potential to become para-extension experts who may professionalize extension services and bring about a paradigm shift in Indian agriculture	5.00	I
Input dealers as a para extension worker helps in selecting beneficiaries to the different agricultural programme	5.00	I
These para extension workers help in planning and implementation of various agricultural programme	5.00	I
A client-cantered approach to extension work that prioritizes the requirements and preferences of specific farmers will not be encouraged by the programme.	4.80	IV
Before the DAESI programme, farmers were not encouraged tobring samples in order to get a solution, but dealers are now requesting that they do.	4.80	IV
To offer extension services to the farming community, separate extension corners have been established inside the presently operating unit.	4.60	VI
The programme introduces digital tools and technologies (DVD, CD and T.V. etc.,) that dealers can use in their shops to enhance their extension services and reach a wider audience	4.60	VI
The programme includes training on interacting with farmers, understanding their needs, and building trust, which are essential qualities of a para-extension worker	4.40	VIII
Input dealers coordinate with agricultural and allied departments and agricultural universities in engaging extension educational activities	4.00	IX

Overall perception of DEASI input dealers on DAESI programme.

The results presented in Table 3 reveals that more than half (56.00 %) of the DAESI input dealers in KVK had good perception towards DAESI programme, whereas 40.00 and 4.00 per cent of input dealers had better and poor level of perception on DAESI programme. DAESI programme gives dealers an in-depth knowledge on agricultural subjects, such as soil science, crop improvement, and pest control. Their expanded knowledge base enables them to provide farmers with better-informed guidance, hence a vast majority of the of the DAESI input dealers had good to better perception towards DAESI programme. These similar results were also reported by Balasubramani (2018), Ganiger (2012) and Ingale (2014).

Relationship between profile characteristics and perception of DAESI input dealers towards programme

From the data in Table 4, it is observed that innovativeness, decision-making and risk-taking ability had strong positive correlation perception of DAESI input dealers towards DAESI programme with 1 per cent level of significance. This demonstrates

TABLE 3

Perception of DAESI input dealers on DAESI programme

(n=50)

Perception categories	DAESI input dealers		Mean	Standard deviation
	Number	Per cent		
Poor < (164.73 score)	2	4.00	181.06	27.15
Good (164.73-194.63 score)	28	56.00		
Better > (194.63 score)	20	40.00		
Total	50	100.00		

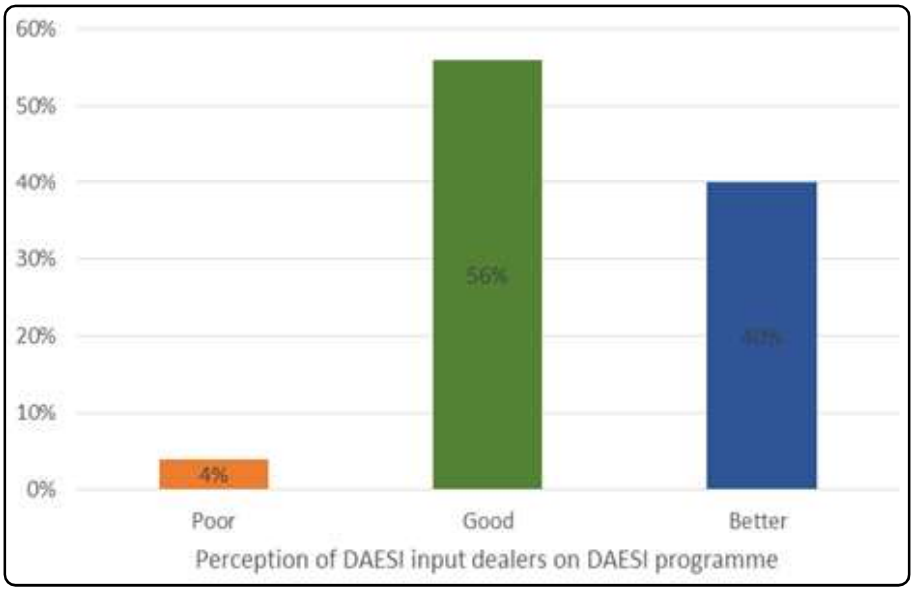

Fig. 1 : Overall perception of DEASI input dealers on DAESI programme

TABLE 4

Relationship between profile characteristics and perception of DAESI input dealers towards programme

Profile characteristics	Correlation coefficient ('r' values)
Age	0.12 NS
Education	0.23 NS
Family background	0.21 NS
Experience in Agri Input dealership	0.15 NS
Annual income	0.19 NS
Type of Agri input sold	0.24 NS
Type of dealership	0.21 NS
Self confidence	0.25 NS
Decision making	1.87 **
Achievement motivation	0.29 *
Leadership abilities	0.98 *
Scientific orientation	0.31 *
Innovativeness	0.89 **
Entrepreneurial behavior	0.28 *
Risk taking ability	0.96 **
Social participation	0.33 *
Extension participation	0.28 *
Mass media exposure	0.34 *

NS= Non-significant; *= Significant at 5%;
**= Significant at 1%

that dealers who are prepared to experiment with new ideas, take calculated risks and make autonomous decisions are better positioned to appreciate and utilize the knowledge and skills imparted by DAESI programme. Whereas, leadership abilities, achievement motivation, scientific orientation, entrepreneurial behavior, extension participation, social participation and mass media exposure also had positive and significantly related with perception of DAESI input dealers towards DAESI programme at 5 per cent level of significance. This suggests that dealers with open-minded, participatory and entrepreneurial attitudes are more approachable to DAESI programme. Such individuals are not only motivated to improve their business practices but also to extend advisory services to farmers, thereby fulfilling the programme's objective of creating para-extension professionals. These results were in line with the results of Ganiger (2012) and Ingale (2014).

Extent of Contribution of Profile Characteristics on Perception of DAESI Input Dealers towards DAESI Programme

Among the eighteen profile characteristics of DAESI input dealers variables such as achievement motivation, social participation, extension participation, scientific orientation, mass media exposure, entrepreneurial behaviour, decision making, leadership abilities, innovativeness and risk taking ability were found to be significant to highly significant with perception of DAESI input dealers towards DAESI programme. All the 18 independent variables have contributed to the tune of 58.10 per cent ($R^2 = 0.581$) in developing better perception of DAESI input dealers towards DAESI programme. The above findings are in conformity with the findings of study conducted by Latha (2019).

TABLE 5

Extent of contribution of profile characteristics on perception of DAESI input dealers towards DAESI programme

(n=50)

Profile characteristics	Regression coefficient	SE (b)	't' value
Age	0.581	0.319	0.549 NS
Education	0.650	0.511	0.786 NS
Family background	0.269	0.127	0.472 NS
Experience in Agri Input dealership	0.102	0.019	0.186 NS
Annual income	0.268	0.200	0.746 NS
Type of Agri input sold	0.155	0.290	1.871 NS
Type of dealership	0.130	0.122	0.938 NS
Self confidence	0.898	0.962	1.071 NS
Decision making	0.292	0.910	3.116 **
Achievement motivation	0.342	0.811	2.371 *
Leadership abilities	0.130	0.578	4.188 **
Scientific orientation	0.277	0.662	2.389 *
Innovativeness	0.291	0.789	2.710 **
Entrepreneurial behaviour	0.317	0.761	2.400 *
Risk taking ability	0.241	0.816	3.385 **
Social participation	0.321	0.715	2.227 *
Extension participation	0.277	0.662	2.389 *
Mass media exposure	0.272	0.710	2.610 *

R2 = 0.581

NS= Non-significant; * = Significant at 5%; ** = Significant at 1%

The perception scale developed is found to be reliable and valid, hence it can be used to analyze the perception of DAESI input dealers on DAESI programme. The perception scale when administered to the DAESI input dealers revealed that majority of the DAESI input dealers (96.0%) had good to better perception towards DAESI programme. This indicates the importance of DAESI programme in training the input dealers to serve as better para extension workers. Decision-making, innovativeness and risk-taking showed a strong positive correlation with perception of DAESI input dealers towards DAESI programme at 1 per cent significance level. Other traits like leadership, motivation, scientific orientation and social participation were also positively related at 5 per cent level. Thus, entrepreneurial and progressive dealers with participatory attitudes tend to view DAESI more favourably.

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Quorum Quenching Potential of *Lactobacillus* sp. : Screening and Characterization from Dairy Products

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Abstract

Quorum sensing (QS) is a bacterial communication mechanism that regulates gene expression in response to population density through signaling molecules such as acyl homoserine lactones (AHLs). Disruption of this process, known as quorum quenching (QQ), offers a promising approach to control bacterial biofilm formation, which is a major concern in milk storage and dairy processing systems. The present study aimed to isolate and characterize lactic acid bacteria (LAB) from various dairy products and evaluate their ability to interfere with QS in *Chromobacterium violaceum*, a biosensor organism used for detecting AHLs. Several LAB isolates exhibited strong QQ activity, as evidenced by a reduction in violacein pigment production and the appearance of inhibition zones in soft agar and disc diffusion assays. Phylogenetic analysis based on 16S rRNA gene sequences identified these isolates as *Lactobacillus* spp. The observed QS inhibition indicates that these LAB strains can disrupt AHL-mediated communication, confirming their potential as quorum-quenching agents. This study highlights the novel application of naturally occurring LAB as eco-friendly biocontrol organisms capable of mitigating QS-regulated biofilm formation. Such LAB-based approaches could contribute to improving food hygiene, extending the shelf life of dairy products and enhancing overall safety in dairy industry environments.

Keywords : Quorum sensing, Screening, Dairy products

Quorum sensing (QS) is a mechanism used by many bacteria to communicate and coordinate their collective behavior through small signaling molecules known as autoinducers (AI). As the bacterial population grows, the concentration of these molecules increases and once a threshold level is reached, QS triggers the expression of genes responsible for virulence, biofilm formation and other physiological processes associated with infection and spoilage.

Quorum quenching (QQ) refers to the disruption of this signaling process by degrading or interfering with the AI molecules, thereby preventing the bacteria from communicating and expressing virulence factors. QQ can effectively reduce the production of harmful metabolites and biofilms, rendering bacteria less pathogenic (Smith *et al*., 2004). Quorum sensing inhibition by QQ bacteria occurs primarily through enzymes capable of degrading QS signaling molecules, such as lactonases, acylases, or oxidoreductases (Sowmiya *et al*., 2022).

Lactobacillus is a genus of lactic acid bacteria (LAB) widely used in fermented food production and probiotic formulations. These bacteria exhibit several beneficial effects, including the production of antimicrobial compounds, enhancement of digestion and maintenance of gut microbial balance. Certain *Lactobacillus* species, such as *L. plantarum* and

L. fermentum, have been reported to exhibit QQ activity by producing enzymes that degrade acyl homoserine lactones (AHLs), the signaling molecules involved in QS (Prazdnova *et al*., 2022).

Dairy-based LAB are particularly promising candidates for QQ research due to their Generally Recognized As Safe (GRAS) status, natural presence in food systems and ability to survive under diverse environmental conditions. Unlike environmental or pathogenic isolates, LAB from fermented dairy products are already well-adapted to food matrices, making them ideal for potential applications in biocontrol and food preservation. Several studies from different regions have demonstrated QQ activity in LAB, highlighting their global potential in mitigating QS-mediated spoilage and biofilm formation in food environments (Salman *et al*., 2023).

Therefore, the present investigation was carried out to isolate and characterize LAB from different dairy products and to evaluate their quorum quenching activity using the biosensor strain *Chromobacterium violaceum*.

Material and Methods

Samples such as raw milk, curd, cheese, yoghurt and buttermilk were collected from locations in the vicinity of GKVK, Bengaluru.

Isolation of Lactic Acid Bacteria from Dairy Products

Lactobacillus Selection Broth Base (M1166) was procured from HiMedia. This medium is recommended for the isolation and enumeration of *Lactobacillus* spp. from food and dairy samples. Isolation of *Lactobacillus* spp. was carried out from raw milk, curd, cheese, yoghurt and buttermilk. Serial dilutions were prepared by adding 10 mL of each sample to 90 mL of sterile distilled water, creating dilutions from 10^{-1} to 10^{-1}. Subsequently, 0.1 mL from the final dilution was spread onto *Lactobacillus* Selection Agar plates. The inoculated plates were incubated at 37 °C for 48 hours (Yashaswini *et al*., 2024). Following growth, morphologically distinct colonies were subcultured and purified by streaking onto fresh *Lactobacillus* Selection Agar plates using sterile inoculating needles. These plates were then incubated at 37 °C for 24-48 hours.

Screening of Quorum Quenching Activty

Violacein Inhibition Assay

A total of fifty-four LAB isolates were tested for their QQ ability using a violacein inhibition assay. Overnight-grown *Chromobacterium violaceum* and bacterial isolates were adjusted to OD_{600} = 0.1 using phosphate buffer. Each LAB isolate's cell-free supernatant was co-inoculated with *C. violaceum* (0.1% each). The bioindicator strain *C. violaceum* alone at 0.1 per cent served as the negative control. All samples were incubated at 30 °C on a rotary shaker (150 rpm) for 24-36 h. Violacein was quantified spectrophotometrically at 585 nm and inhibition was calculated as described by (Sowmiya *et al*., 2023) using the following formula:

$$\text{Violacein inhibition percentage (\%)} = \frac{OD_{585}\ \text{control} - OD_{585}\ \text{sample}}{OD_{585}\ \text{control}} \times 100$$

Standardization of Procedure for Screening the Bacterial Isolates for AHLase Activity

The five most efficient bacterial isolates (UAS LAB 6, UAS LAB 10, UAS LAB 14, UAS LAB 19 and UAS LAB 33) obtained from the violacein inhibition assay were screened for quorum-quenching activity using the biosensor strain *C. violaceum* (CV). Two screening methods were used: the soft agar diffusion assay (McLean *et al*., 2004) and the disc diffusion assay (Song *et al*., 2012), both to detect enzyme activity against AHLs.

Soft Agar Diffusion Assay

This assay was performed with slight modification from (Packialakshmi *et al*., 2021). Overnight-grown *C. violaceum* was plated on tryptic soy agar medium. Wells (6 mm diameter) were bored using a sterile cork borer, and 100 µL of cell-free supernatant from LAB cultures was added to each well. Plates were incubated at 30 °C for 24 h. Clear zones around the wells

indicated degradation of the purple violacein pigment. A heat-inactivated supernatant served as a control to confirm that pigment inhibition was enzyme-mediated.

Disc Diffusion Assay

Disc diffusion was performed on double-layer agar plates. The basal layer contained LB medium with 2 per cent agar, and the top layer contained LB medium with 0.5 per cent agar seeded with 5 per cent *C. violaceum* (v/v). Cell-free supernatants of overnight-grown LAB were obtained by centrifugation (8000 rpm, 10 min) and filtration through 0.2 µm membranes. Sterile 6 mm filter paper discs were soaked in the supernatant for 30 s and placed on the plates. Plates were incubated at 30 °C for 24 h and anti-quorum sensing activity was identified by the presence of clear zones around discs (Packialakshmi *et al.*, 2021). A heat-inactivated supernatant served as a control to confirm that pigment inhibition was enzyme-mediated.

Screening of the Isolates for QQ Ability

The five efficient isolates were evaluated for anti-quorum sensing ability using the standardized assays above. Plates were incubated at 30 °C and the diameters of colonies and zones of clearance were measured. *Prestia aryabhattai* (Accession No. OL662868) served as the positive control. The pigmentation loss index (PLI) and QQ efficiency (%) were calculated as follows.

$$\text{Pigmentation loss Index (PLI)} = \frac{\textit{well diameter}}{\textit{Zone diameter}} \times 100$$

$$\text{QQ efficiency \%} = \frac{\textit{Total diameter - well diameter}}{\textit{Total diameter}} \times 100$$

Characterization of Lactic Acid Bacterial Isolates

Morphological Characterization

The five efficient LAB isolates were examined for colony morphology, including form, pigmentation, elevation and margin. Cell morphology was observed by simple staining using 48-hour-old cultures. Smears were stained with crystal violet for one minute, rinsed, air-dried and examined under 100X magnification (Becking, 1974).

Gram Reaction

The five efficient lactic acid bacterial (LAB) isolates were subjected to Gram reaction. A heat fixed bacterial smear was first stained with a primary stain (crystal violet), then a mordant (Gram's Iodine), then a quick decolorization with alcohol and finally a counter stain (safranin). Each stain was applied, left on the smear for a minute and then rinsed off before the next stain was applied except safranin. The counter stain was left for 30 sec. and washed to eliminate any remaining colour. The stained bacterial smear was observed under 100 X magnification (Olympus CX21iLEDFS1) (Bartholomew and Mittwer, 1952).

Biochemical Characterization

Catalase Activity

The catalase test facilitates the detection of the enzyme catalase in bacteria. A loop full of test cultures was placed on a clean slide and 3 per cent hydrogen peroxide was added. Effervescence production indicating a positive result for the catalase test (Dhameliya *et al*., 2020).

Acid and Gas Production

The glucose broth with pH indicator (phenol red) and along with an inverted Durham's tube in a test tube were sterilized and inoculated with 48 h old bacterial isolates to observe for acid and gas production. These tubes were incubated for two days at 32 °C to observe a colour change from red to yellow (due to acid production) and the appearance of bubbles in Durham's tube (due to gas production) (Seeley and Vandemark, 1970).

Molecular Characterization

DNA Extraction

The genomic DNA of the bacterial isolates was extracted by alkaline lysis method (Feliciello and Chinali, 1993). The bacterial isolates were grown in nutrient broth for 48 hours at 30 °C and centrifuged 3

mL of culture at 12,000 rpm for 3 min, bacterial pellets were obtained, pellet was resuspended in 650 µL of extraction buffer and incubated at 65 °C for 30 min. 100 µL of 5 M potassium acetate solution was added to the extract and allowed for precipitation of protein and carbohydrates by placing it on ice for 15 min. The supernatant was collected by centrifuging at 12,000 rpm for 8 min. The DNA was precipitated by adding an equal volume of ice-cold isopropanol and pelleted at 12,000 rpm for 5 min. The pellet obtained was washed twice with 70 per cent ethanol, air-dried and dissolved in 1mM TE (10:1). The concentration of DNA was measured using UV spectrometer at 260 /280 nm wavelength and stored at -20 °C.

PCR Amplification of the DNA

The two primers (22 bp forward primer 5'-GGAGAGTTAGATCTTGGCTCAG-3' and 20 bp reverse primer 5'-AAGGAGGGGATCCAGCCGCA-3'), previously reported for amplification of 16S rRNA gene sequences (Weisburg *et al*., 1991), were custom synthesized by Sigma-Aldrich (Sigma, USA) and diluted accordingly for PCR reactions using DNA isolated from bacterial isolates.

The annealing temperature for primer pair was standardized and PCR was run in 40 µL reaction mixture containing 1X buffer with $MgCl_2$ (1.5 mM), dNTPs (200 µM), forward and reverse primers (0.5 µM each), *Taq* DNA polymerase and 2 µL template DNA (50 ng /µL). The amplification was carried out with an initial denaturation at 96 °C for four minutes followed by 35 amplification cycles consisting of 94 °C for one minute, 55 °C for 30 seconds and 72 °C for one minute and a final extension at 72 °C for 12 min. The controls for PCR reactions were carried out with the same primers without providing template DNA (Yurgel *et al*., 2017).

Agarose Gel Electrophoresis

Agarose gel electrophoresis was performed to resolve the amplified product using 0.8 per cent Agarose in 1X TAE buffer, 0.5 µg /mL of ethidium bromide and loading buffer (0.25% bromophenol blue prepared in 40% sucrose). Five µL of loading dye was added to 40 µL of PCR product and loaded to the well. Electrophoresis was carried out at 100 V for two hours. The gel was visualized under a UV transilluminator and documented using a gel documentation unit (Comeau *et al*., 2016).

Gel Elution

The gene JETTM Gel extraction kit (Thermo Scientific) was used for rapid and efficient separation of DNA fragments from agarose gel. The gel slice containing DNA was excised using a clean razor blade and was placed into a pre-weighed 1.5 mL tube. An equal volume of binding buffer (100 mg gel slice: 100 µL of binding buffer) was added to the tube. The mixture was incubated at 60 °C till the gel slice was completely dissolved. The solubilized gel solution was added to the Gene JETTM purification column and centrifuged at 10,000 rpm for 60 sec. The flow-through was discarded and the column was placed back into the same collection tube washed by adding 700 µL of wash buffer by centrifugation at 10,000 rpm for 60 sec. The tube was again centrifuged for 60 seconds to remove the wash buffer that remained as residue. Then purification column was placed into a clean 1.5 mL microcentrifuge tube and added with 20 µL of elution buffer and centrifuged at 10,000 rpm for 60 sec. The eluted DNA was checked for its concentration using nanodrop and got it sequenced by Barcode Bio Sciences, Bengaluru, Karnataka. The sequence data received from the company was analyzed for homology.

Sequence Analysis and Homology Search

The sequence analysis and homology search sequence results were analyzed using online software from National Centre for Biotechnology Information (NCBI). The sequence was aligned by CLUSTAL W multiple sequence alignment program. A phylogenetic tree was constructed with bootstrap support based on a neighbor-joining analysis in MEGA11 version 11.0.13 and DARwin version 6.0.021.

Results and Discussion

Dairy samples were collected from the Zonal Agricultural Research Station (ZARS), local supermarkets and nearby farmers in the vicinity of Gandhi Krishi Vignana Kendra, College of Agriculture, University of Agricultural Sciences, Bangalore. The samples were subjected to serial dilution and plated on Lactobacillus Selection Agar (HiMedia). Colonies exhibiting typical lactic acid bacterial (LAB) morphology were transferred to fresh LAB selective agar plates for further isolation. A total of 54 morphologically distinct colonies were isolated and purified for screening their quorum quenching potential.

The quorum quenching (QQ) potential of all 54 isolates was quantitatively evaluated using the violacein inhibition assay against *Chromobacterium violaceum* (MCC 4212), a widely employed biosensor for *N*-acyl homoserine lactone (AHL)-mediated quorum sensing. Among the isolates, UAS LAB 6 exhibited the highest level of inhibition (60.15%), followed by UAS LAB 10 (56.95%), UAS LAB 33 (54.83%), UAS LAB 19 (48.49%) and UAS LAB 15 (43.39%) (Table 1). These findings indicate notable QQ activity, potentially attributed to the production of quorum quenching enzymes capable of degrading AHLs. Comparable levels of violacein inhibition have been reported in *Lactobacillus* species by Balamurugan *et al*. (2020), who observed over 50 per cent inhibition in *L. plantarum* and *L. fermentum* strains isolated from fermented foods.

In contrast, isolates such as UAS LAB 38 (0.06%) and UAS LAB 35 (0.26%) demonstrated negligible violacein inhibition, suggesting a lack or minimal presence of QQ activity. This variability in QQ potential among LAB isolates has also been reported by Kim *et al.* (2018), who proposed that QQ capability is highly strain-specific and influenced by environmental adaptation and metabolic versatility.

All experiments were performed in triplicate and data were expressed as mean ± standard deviation (SD). Statistical analysis of the inhibition data was carried out using one-way ANOVA followed by

Table 1

Inhibition of violacein production by 54 LAB isolates by using *Chromobacterium violaceum* as a biosensor in volacein inhibition assay

Isolates code	Inhibition
UAS LAB 1	0.00 ± 0.00 ^{D} (0.05)
UAS LAB 2	0.09 ± 0.00 ^{qr} (0.31)
UAS LAB 3	0.10 ± 0.00 ^{q} (0.32)
UAS LAB 4	0.42 ± 0.02 ^{f} (0.70)
UAS LAB 5	0.24 ± 0.00 ^{ij} (0.51)
UAS LAB 6	0.60 ± 0.01 ^{a} (0.89)
UAS LAB 7	0.00 ± 0.00 ^{D} (0.05)
UAS LAB 8	0.22 ± 0.00 ^{l} (0.49)
UAS LAB 9	0.04 ± 0.00 ^{v} (0.21)
UAS LAB 10	0.57 ± 0.02 ^{b} (0.86)
UAS LAB 11	0.28 ± 0.00 ^{h} (0.56)
UAS LAB 12	0.03 ± 0.00 ^{yz} (0.16)
UAS LAB 13	0.20 ± 0.00 ^{m} (0.46)
UAS LAB 14	0.04 ± 0.00 ^{w} (0.19)
UAS LAB 15	0.43 ± 0.02 ^{e} (0.72)
UAS LAB 16	0.23 ± 0.00 ^{jk} (0.50)
UAS LAB 17	0.03 ± 0.00 ^{wx} (0.18)
UAS LAB 18	0.18 ± 0.00 ^{n} (0.44)
UAS LAB 19	0.48 ± 0.02 ^{d} (0.77)
UAS LAB 20	0.09 ± 0.00 ^{r} (0.30)
UAS LAB 21	0.02 ± 0.00 ^{zA} (0.16)
UAS LAB 22	0.21 ± 0.01 ^{m} (0.47)
UAS LAB 23	0.03 ± 0.00 ^{yz} (0.16)
UAS LAB 24	0.43 ± 0.01 ^{e} (0.72)
UAS LAB 25	0.01 ± 0.00 ^{C} (0.10)
UAS LAB 26	0.07 ± 0.00 ^{stu} (0.27)
UAS LAB 27	0.02 ± 0.00 ^{B} (0.13)
UAS LAB 28	0.10 ± 0.00 ^{qr} (0.32)
UAS LAB 29	0.00 ± 0.00 ^{D} (0.05)
UAS LAB 30	0.14 ± 0.01 ^{op} (0.38)
UAS LAB 31	0.07 ± 0.00 ^{s} (0.27)
UAS LAB 32	0.13 ± 0.00 ^{p} (0.37)
UAS LAB 33	0.55 ± 0.02 ^{c} (0.83)

Continued....

Table 1 Continued....

Isolates code	Inhibition
UAS LAB 34	0.14 ± 0.00 ^{o} (0.39)
UAS LAB 35	0.00 ± 0.00 ^{D} (0.05)
UAS LAB 36	0.02 ± 0.00 ^{A} (0.15)
UAS LAB 37	0.07 ± 0.00 st (0.27)
UAS LAB 38	0.00 ± 0.00 ^{E} (0.03)
UAS LAB 39	0.25 ± 0.00 ^{i} (0.52)
UAS LAB 40	0.09 ± 0.00 ^{r} (0.31)
UAS LAB 41	0.04 ± 0.00 ^{v} (0.21)
UAS LAB 42	0.23 ± 0.01 ^{kl} (0.49)
UAS LAB 43	0.03 ± 0.00 ^{xy} (0.17)
UAS LAB 44	0.04 ± 0.00 ^{w} (0.19)
UAS LAB 45	0.14 ± 0.01 ^{o} (0.39)
UAS LAB 46	0.38 ± 0.01 ^{g} (0.67)
UAS LAB 47	0.07 ± 0.00 ^{stu} (0.27)
UAS LAB 48	0.13 ± 0.00 ^{p} (0.37)
UAS LAB 49	0.03 ± 0.00 ^{xy} (0.17)
UAS LAB 50	0.07 ± 0.00 ^{stu} (0.26)
UAS LAB 51	0.03 ± 0.00 ^{yz} (0.17)
UAS LAB 52	0.07 ± 0.00 ^{u} (0.26)
UAS LAB 53	0.07 ± 0.00 ^{tu} (0.26)
UAS LAB 54	0.03 ± 0.00 ^{wx} (0.18)

Note : Cell values are mean ± SD; Transformed mean in parenthesis

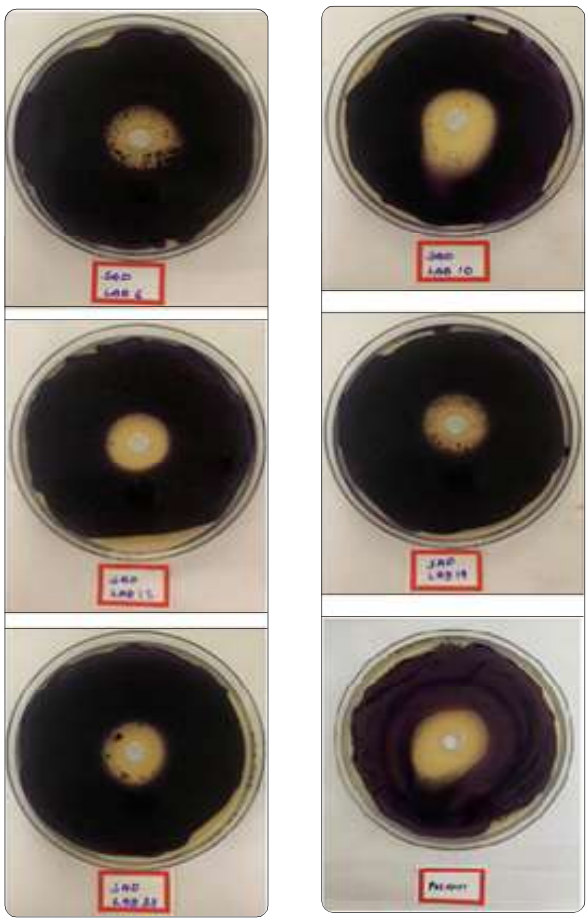

Plate 1 : Quorum quenching potential of LAB against *Chromo bacterium violaceum* as determined by soft agar diffusion method Positive/ Reference culture: *Prestia aryabhattai*

Duncan's multiple range test ($p < 0.05$) to determine significant differences among isolates.

These findings are in agreement with earlier studies by Wang *et al*. (2017) and Patel and Thakkar (2015), who highlighted the largely unexplored potential of traditional dairy sources for isolating bioactive microbial strains. The five efficient QQ isolates were further evaluated for their quorum quenching potential using both soft agar diffusion and disc diffusion assays, employing *Chromobacterium violaceum* (MCC 4212) as the bioindicator organism (Plate 1 and Plate 2). Data on QQ activity, including zone of inhibition, Pigmentation Loss Index (PLI) and overall QQ efficiency, as assessed through the soft agar diffusion assay and disc diffusion assays, are presented in Table 2. Among the five isolates tested, UAS LAB 6 demonstrated the highest zone of inhibition (1.73 ± 0.03 cm), followed by UAS LAB 10 (1.63 ± 0.03 cm) and UAS LAB 15 (1.37 ± 0.05 cm). These results were benchmarked against the reference QQ strain *Prestia aryabhattai*, which exhibited a zone diameter of 1.89 ± 0.05 cm.

The Pigmentation Loss Index (PLI), an indicator of violacein pigment suppression, ranged from 0.17 to 0.24 across the tested isolates. UAS LAB 33 exhibited the highest PLI value (0.24), indicating significant interference with AHL-mediated signalling, whereas UAS LAB 19 showed the lowest PLI (0.17). Quorum quenching efficiency was greatest in UAS LAB 6 (82.65%), closely followed by UAS LAB 10 (81.59%). All the tested isolates

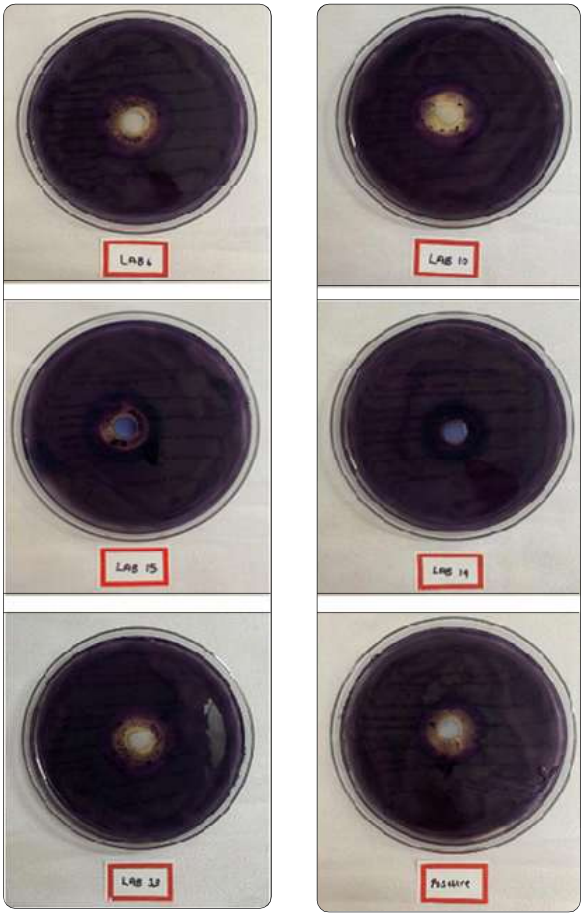

Plate 2 : Quorum quenching potential of LAB against *Chromo bacterium violaceum* as determined by disc diffusion assay
Positive/ Reference culture: *Prestia aryabhattai*

have potential to disrupt quorum sensing mechanisms. Similar trends were observed by Balamurugan *et al*. (2020), who reported PLI values exceeding 0.5 in *Lactobacillus fermentum*, correlating with pronounced anti-quorum sensing activity. The consistency of results across violacein inhibition, diffusion assays and PLI values confirms the robustness of QQ activity exhibited by the selected LAB isolates. Further, the isolate UAS LAB 6, which displayed the highest inhibition (60.15%) in the violacein assay, also exhibited the largest zone of inhibition, reinforcing its consistent and robust QQ activity across different assay methodologies. These findings align with observations by Wang *et al*. (2017), who reported that effective QQ strains tend to maintain activity across multiple *in vitro* assay platforms. The soft agar diffusion method enabled visualisation of diffusible pigment inhibition surrounding the wells, whereas the disc diffusion assay provided more standardised and quantifiable zone measurements. This multi-assay approach minimized false positives arising from general growth inhibition, as confirmed by zones showing pigment loss without microbial lysis (Ahmad *et al*., 2019).

The combined use of qualitative and quantitative bioassays enhanced result reliability by assessing signal disruption from multiple perspectives: inhibition zones confirmed diffusible inhibitory compounds, while pigment suppression directly demonstrated AHL-interference (Kim *et al*., 2018).

Comparable methodologies have been effectively utilised in prior studies for the isolation of lactic acid bacteria (LAB) from milk and dairy products (Kumar *et al*., 2017). The colony morphologies observed

Table 2

Quorum Quenching (QQ) Activity of LAB against *C. violaceum*

LAB Isolates	Zone diameter (cm)	Pigmentation lossIndex (PLI)	QQ Efficiency (%)
UAS LAB 6	1.73 ± 0.06 ^{b}	0.17 ± 0.01 ^{d}	82.65 ± 3.42 ^{a}
UAS LAB 10	1.63 ± 0.02 ^{c}	0.18 ± 0.01 ^{c}	81.59 ± 0.81 ^{ab}
UAS LAB 14	1.37 ± 0.00 ^{d}	0.21 ± 0.00 ^{b}	78.10 ± 2.46 ^{bc}
UAS LAB 19	1.40 ± 0.06 ^{d}	0.21 ± 0.00 ^{b}	78.57 ± 0.07 ^{bc}
UAS LAB 33	1.27 ± 0.02 ^{e}	0.24 ± 0.01 ^{a}	76.37 ± 2.20 ^{c}
Reference strain (*Prestia aryabhattai)*	1.89 ± 0.07 ^{a}	0.16 ± 0.00 ^{e}	84.12 ± 2.28 ^{a}

TABLE 3

Colony and cell morphological characteristics of lactic acid (LA) bacterial isolates

Isolate code	Colony Morphology				Cell Morphology	
	Form	Pigmentation	Elevation	Margin	Shape	Arrangement
UAS LAB 6	Small circular	Bright white	Raised	Entire	Rods	Single
UAS LAB 10	Small circular	Bright white	Raised	Entire	Rods	Single
UAS LAB 15	Small circular	Bright white	Raised	Entire	Rods	Single
UAS LAB 19	Small circular	Bright white	Raised	Entire	Rods	Single
UAS LAB 33	Small circular	Bright white	Raised	Entire	Rods	Single

TABLE 4

Staining and biochemical characteristics of LAB isolates

Isolate code	Gram's reaction	Catalase activity	Acid production	Gas production
UAS LAB 6	+	-	+	-
UAS LAB 10	+	-	+	-
UAS LAB 15	+	-	+	-
UAS LAB 19	+	-	+	-
UAS LAB 33	+	-	+	-

Note : '+'-positive,'-'–negative

circular and white are consistent with the typical characteristics reported for *Lactobacillus* species and other LAB genera (Joshi and Sharma, 2018).

Microscopic examination revealed rod-shaped bacteria, arranged singly, aligning with the morphological traits commonly associated with LAB, as documented by Ahmad *et al*. (2019). The observed cell morphology among isolates suggests the presence of LAB population within the samples. Additionally, the Gram-positive reaction and cata lase-negative activity of the isolates further confirmed their classification as LAB (Verma *et al.*, 2016).

Biochemical characterization revealed inter-strain variability in catalase activity, gas and acid production, consistent with the observations of Patel and Thakkar (2015). Such diversity highlights potential differences in enzymatic systems responsible for quorum quenching and other probiotic properties (Lee *et al.*, 2020).

To complement the phenotypic and functional characterisation, molecular identification of the top five efficient quorum quenching (QQ) LAB isolates was carried out through 16S rRNA gene sequencing. BLAST analysis of the resulting sequences against the NCBI database indicated a

TABLE 5

Molecular identification of quorum quenching LAB isolates based on 16S rRNA gene sequencing

Isolate	Organism	Percentage similarity (%)	Accession number
UAS LAB 6	*Limosilactobacillus fermentum*	98.83	PV687706
UAS LAB 10	*Limosilactobacillus fermentum*	99.60	PV687707
UAS LAB 15	*Lactiplantibacillus plantarum*	98.49	PV627910
UAS LAB 19	*Limosilactobacillus fermentum*	98.60	PV687692
UAS LAB 33	*Lactiplantibacillus plantarum*	98.05	PV687693

high degree of similarity (≥98%) with recognised LAB species, thereby validating the earlier morphological and biochemical classifications. This molecular approach is widely regarded as a reliable method for LAB identification and has proven effective in differentiating even closely related strains (Moura *et al*., 2020).

Using the NCBI database and the BLAST programme, five obtained sequences were analysed (Table 5). Nucleotide sequence alignment was performed using the Molecular Evolutionary Genetics Analysis tool. The aligned sequences served as the input for constructing a phylogenetic tree, generated using the Jukes-Cantor model with a bootstrap value of 1000, via the Dissimilarity Analysis and Representation for Windows software. The resulting phylogenetic relationships are illustrated in Fig. 1. Isolates LAB15 and LAB33 formed a distinct clade with *Lactiplanti bacillus plantarum* strain NRRL B-14768, supported by a bootstrap value of 80-100 per cent, indicating a close genetic relationship. Similarly, isolates LAB6, LAB10 and LAB19 grouped with *Limosilacto bacillus fermentum* and *Limosilactobacillus reuteri* sub sp. *reuteri* with high bootstrap support (75-100%). The tree also showed close clustering of reference species including *Lactobacillus brevis, Lactobacillus johnsonii* and *Lactobacillus acidophilus,* confirming the genetic diversity among QQ Lactic acid bacteria isolates. The percentage sequence similarity between the isolates and their closest related type strains ranged from 97.50 to 99.80 per cent, indicating that the isolates belong to known *Lactobacillus* and *Limosilactobacillus* species. These associations are consistent with previous studies that have frequently identified these species in dairy environments with

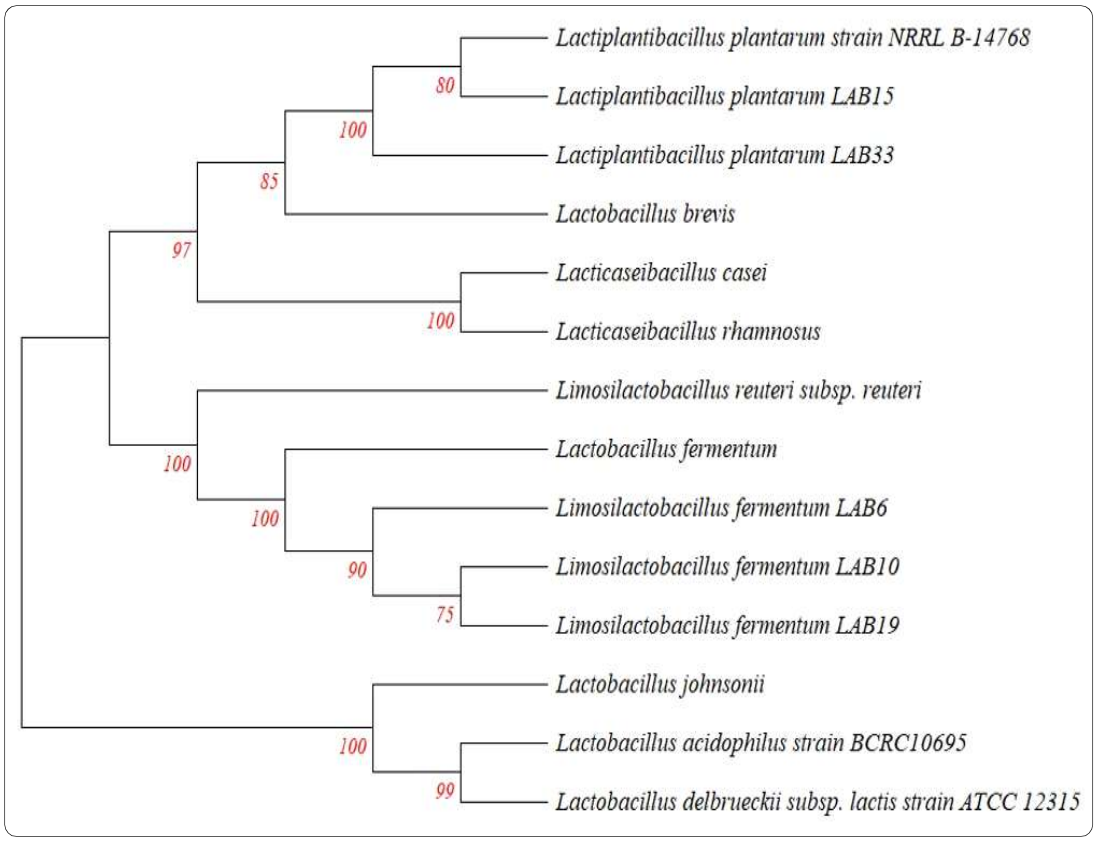

Fig. 1 : Phylogenetic tree showing the relationship of quorum quenching (QQ) LAB isolates with previously reported QQ bacterial strains based on 16S rRNA gene sequences

established quorum quenching potential (Kaur *et al*., 2021 and Wang *et al*., 2017).

The use of DARwin 6 software enabled high-resolution visualization of dissimilarity-based clustering, complementing distance-based phylogenetic methods. Phylogenetic analysis confirmed taxonomic identities and suggested possible evolutionary conservation of QQ-related genes, potentially influenced by horizontal gene transfer (Tashiro *et al.*, 2016).

The preliminary screening provides a basis for subsequent molecular identification and functional characterisation of quorum quenching activity. Research conducted by Balamurugan *et al.* (2020) demonstrated that certain strains of *Lactobacillus plantarum* and *Lactobacillus fermentum* are capable of disrupting quorum sensing signals in spoilage bacteria, thereby highlighting their potential in probiotic development and quorum quenching applications.

Identification of potent QQ strains among dairy-derived LAB presents new opportunities for developing next-generation probiotics that may inhibit QS-regulated spoilage bacteria such as *Pseudomonas* spp. in dairy systems. These LAB can potentially prevent biofilm formation, attenuate virulence and improve the microbiological safety of milk and dairy products even at low temperatures (Lee *et al.*, 2020).

The present study isolated and characterized a total of 54 lactic acid bacteria from a variety of dairy products and identified several strains with notable quorum quenching (QQ) potential. Violacein inhibition assays followed by disc diffusion and well diffusion methods showed that isolates UAS LAB 6, UAS LAB 10, UAS LAB 33, UAS LAB 19 and UAS LAB 15 have significant interference with quorum sensing in *Chromobacterium violaceum*. Morphological, biochemical and molecular characterization further validated their identity as LAB species with recognized bioactive properties. The ability of these strains to disrupt AHL-mediated signaling underscores their potential application in microbial biocontrol, food preservation and probiotic development. This work provides one of the first comprehensive evaluations of dairy-derived lactic acid bacteria from the south India for their quorum-quenching ability, demonstrating that naturally occurring LAB from traditional dairy sources can serve as effective, safe and eco-friendly alternatives to chemical quorum sensing inhibitors. The integration of functional screening with molecular identification offers new insight into the strain-specific basis of QQ activity in LAB, thereby advancing understanding of their dual role as probiotics and QQ agents. Future investigations may focus on enzyme profiling and *in vivo* validation to elucidate their mechanisms of food spoilage inhibition and their contribution to gut health enhancement.

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Formulation and Quality Evaluation of *Nachos* Prepared from Buckwheat (*Fagopyrum esculentum)*

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ABSTRACT

Buckwheat (*Fagopyrum esculentum*), nutrient-dense pseudo-cereal rich in protein, minerals, dietary fibre and phytonutrients, remains underutilized despite its recognized health benefits and gluten-free nature. The present study was undertaken to formulate and evaluate buckwheat-based *nachos* as a value-added ready-to-eat (RTE) snack. Buckwheat flour was incorporated at 25, 50, 75 and 100% levels by replacing maize flour, along with a control (100% maize). Among all treatments, the 100% buckwheat *nachos* (BWN4) recorded the highest sensory acceptability with superior scores for appearance (8.15), colour (8.16), texture (8.37), flavour (8.45), taste (8.26) and overall acceptability (8.43). Proximate analysis revealed that BWN4 had higher protein (13.99 g/100g), fat (19.63 g/100g), ash (1.90 g/100g) and crude fibre (3.06 g/100g) compared to the control, along with enhanced mineral content including calcium (87.58 mg/100g), phosphorus (246.67 mg/100g), potassium (387.49 mg/100g), magnesium (149.51 mg/100g) and iron (4.57 mg/100g). Buckwheat *nachos* also exhibited higher tannins (5.39 mg TAE/100g), phytic acid (314.63mg/100g) and total antioxidant activity (270.48 mg AAE /100g), confirming improved functional properties. Texture analysis indicated significantly lower hardness in BWN4 (7.84 N) compared to the control (13.66 N), contributing to better crispness. Storage studies conducted for 60 days in MPP packaging showed a gradual but acceptable increase in moisture, peroxide value and free fatty acids with increase in storage days and values were within the safe limit for both samples. Sensory scores declined over time but remained within the acceptable range throughout storage. Overall, the study demonstrated that buckwheat can be effectively utilized to develop nutritious, sensory-acceptable and shelf-stable *nachos.* Incorporation of buckwheat at 100% enhances the nutritional, functional and textural qualities of the product, supporting its potential for developing healthier RTE snacks.

Keywords : Buckwheat, Product development, Nutritional composition and Storage studies

PSEUDO-CEREALS are non-grass, wild plants whose seeds are used in the same manner as cereals, but are underutilized due to the dominanace of conventional cereal crops. They are climatically more resilient and nutritionally richer than major cereal crops. They contain adequate amounts of dietary fibers that help improve lipid metabolism. They also contain saponins, polyphenols, betalains, flavonoids, antioxidants, vitamins and other important phytochemical compounds that help detoxify ROS and cope up with the diseases (Nagar *et al*., 2022)

The main pseudo cereals with health-related benefits are buckwheat, amaranth and quinoa. Buckwheat is a plant that belongs to the Polygonaceae family and is used as a pseudo cereal due to its nutritional properties. It has been consumed for thousands of years in many parts of the world.

Buckwheat is known to contain energy (355Kcal), crude protein (12g), carbohydrate (72.9g), total fiber (17.8g), fat (7.4g), moisture (11%). It is rich in various bioactive compounds such as rutin, quercetin, kaempferol-3-rutinoside and therefore increasingly considered a potential functional food. Predominant minerals of buckwheat include potassium, magnesium, phosphorus, calcium, iron, copper, zinc, manganese and vitamins include β - carotene, thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, ascorbic acid and tocopherols (Pirzadah & Malik, 2020 and Puligundla & Lim, 2021). Buckwheat is an excellent source of protein, containing all eight essential amino acids. This makes it a good choice for vegetarians and vegans. Buckwheat is naturally gluten-free, making it a safe and nutritious option for people with celiac disease or gluten intolerance. Buckwheat has a low glycaemic index, which means it can help regulate blood sugar levels and may reduce the risk of developing type 2 diabetes. It is a good source of dietary fiber, which can help promote digestive health and may reduce the risk of heart disease (Sharma & Minhas, 2023).

Buckwheat is a versatile ingredient that can be used in a variety of dishes and it has a unique, nutty flavour that is popular in many cuisines (Sharma and Minhas, 2023). Buckwheat is derived from beech and wheat due to its similarity with beechnut and wheat. Buckwheat has been called different names by different countries and is called as ogal in India (Sofi *et al.* 2023). Buckwheat can be prepared in a variety of ways, such as in pancakes, porridge and noodles. It can also be used to make flour, which is a popular ingredient in gluten-free baking and also in many value added products (Bhinder *et al.* 2022). In recent years, the fast moving and changing lifestyle have also changed eating habits based largely on Ready to Eat (RTE) and convenient foods which resulted in boosting of food processing industry (Rachitha *et al*., 2025). A fast-paced urban lifestyle, increasing dominance of nuclear family structure, rising disposable income, convenience of use has made RTE foods popular. Provision of such RTE foods based on nutritious grains would be more meaningful in the modern times in the management of life style disorders (Takhellamban and Chimmad, 2015).

Nachos, a Mexican dish, are prepared from corn or wheat flour and also known as t*ortilla* chips. *Nachos*, a popular snack can be served with or without sauces and spices based on individual preferences with their rich composition of carbohydrates, protein and fats (Farooqui *et al.* 2023). As consumer demand for healthier and more diverse food options continues to grow, Buckwheat's nutritional richness, unique sensory attributes, there is a need to research about this under-utilized crop and create awareness as this crop has many nutritional and health benefits. The present study was conducted to formulate and evaluate the quality of *nachos* prepared from buckwheat.

Material and Methods

Procurement of Buckwheat

Buckwheat variety Nelagiri was procured from AICRN, Potential crops UAS, GKVK, Bengaluru and was processed in to flour and other ingredients were procured from local market, Bengaluru.

Processing of Buckwheat

Buckwheat is dehulled in (model: UASB-2018, Bengaluru) machine and obtained groats were processed into flour and it is used for the further study.

Nutrient Composition of Buckwheat Flour

All analysis was done by following AOAC (2005) methods. Moisture was determined from sample weight loss after drying in the oven at 105 °C for 2-3 hours. Protein (g) content was determined by kjeldhal method. The Soxhlet method was used for total fat (g) determination. Crude fiber was estimated by using moisture and fat-free samples by treatment of sample first with acid and subsequently with alkali. Micro nutrients such as iron, manganese, zinc and copper was analyzed using Atomic Absorption Spectrophotometer, calcium and magnesium by titration method and phosphorus and potassium was assessed by standard procedure (AOAC, 2005).

Development of *Nachos* from Buckwheat Flour

The *nachos* were standardized by incorporating buckwheat flour with maize flour at 25, 50, 75 and 100 per cent. Control *nachos* were prepared from 100 per cent maize flour. All other ingredients for the preparation of *nachos* like refined wheat flour, turmeric, salt, chilli powder, sesame seeds and ajwain were kept constant for all the variations. The preparation of *nachos* is presented in Fig. 1.

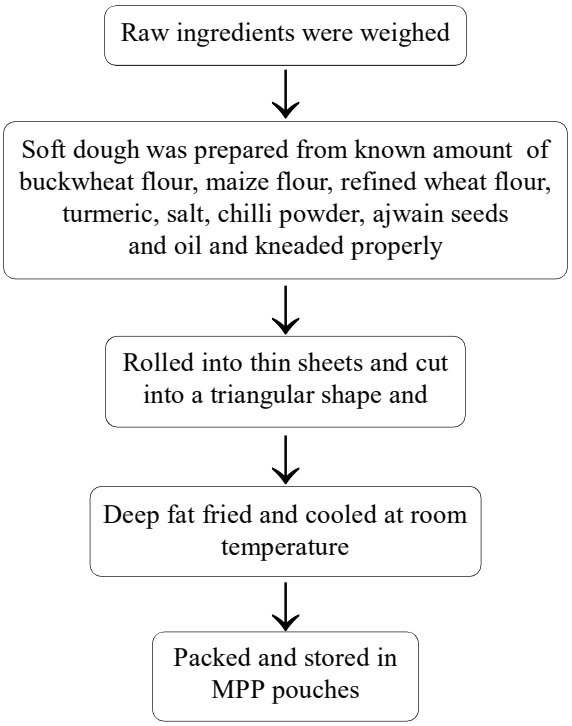

Fig. 1 : Preparation of Buckwheat *Nachos*:

Sensory Evaluation

Developed buckwheat *nachos* variations were evaluated for their sensory attributes using nine-point hedonic scale by a panel of 21 semi trained members. The products were evaluated for sensory attributes such as for the appearance, colour, texture, taste, flavour and overall acceptability.

Nutrient Composition, Phytonutrients and Total Antioxidant Activity of Best Accepted Buckwheat *Nachos*

Best accepted buckwheat *nachos* were analyzed for macronutrient and micronutrient composition using standard protocols.

Phytonutrients and Total Antioxidant Activity of Best Accepted Buckwheat *Nachos*

Best accepted buckwheat *nachos* were analyzed for tannins using calorimetrically based on the measurement of blue color formed by the reduction of phosphotungstomoybdic acid in alkali solution.

$$\text{Tannin as tannic acid \%} = \frac{\text{mg of tannic acid x dilution x 100}}{\text{Aliquot taken for estimation x weight of sample x 100}}$$

In phytic acid phytate is extracted with trichloroacetic acid and precipitated as ferric salt. The iron content of the precipitate was determined calorimetrically and the phytate phosphorus content calculated from this value assuming a constant 4 Fe: 6 molecular ratios in the precipitate.

Phytates were estimated as phytic and phytate phosphorous was obtained.

$$\text{Phytate P (mg/100g sample)} = \frac{\mu g\ Fe \times 15 \times 1}{\text{weight of sample (g)}}$$

Total antioxidant activity was done by using DPPH(2,2-diphenyl-1-picrylhydrazyl) method.

$$\text{Total Antioxidant activity} = \frac{(AB\text{-}AS) \times \text{standard value } (\mu g) \times \text{total volume extracted})}{(\text{Assay volume} \times \text{Weight of sample (g)} \times 1000)}$$

AB = Absorbance of blank sample

AS = Absorbance of testing sample

Texture Analysis of Best Accepted Buckwheat *Nachos*

The hardness profile of the developed products was assessed using a texture analyzer TA-XT plus (Stable Micro Systems).

Storage Study of Buckwheat *Nachos*

Control and buckwheat *nachos* were packed in multi layered polypropylene (MPP) packages and storage stability was carried out for 60 days. The stored

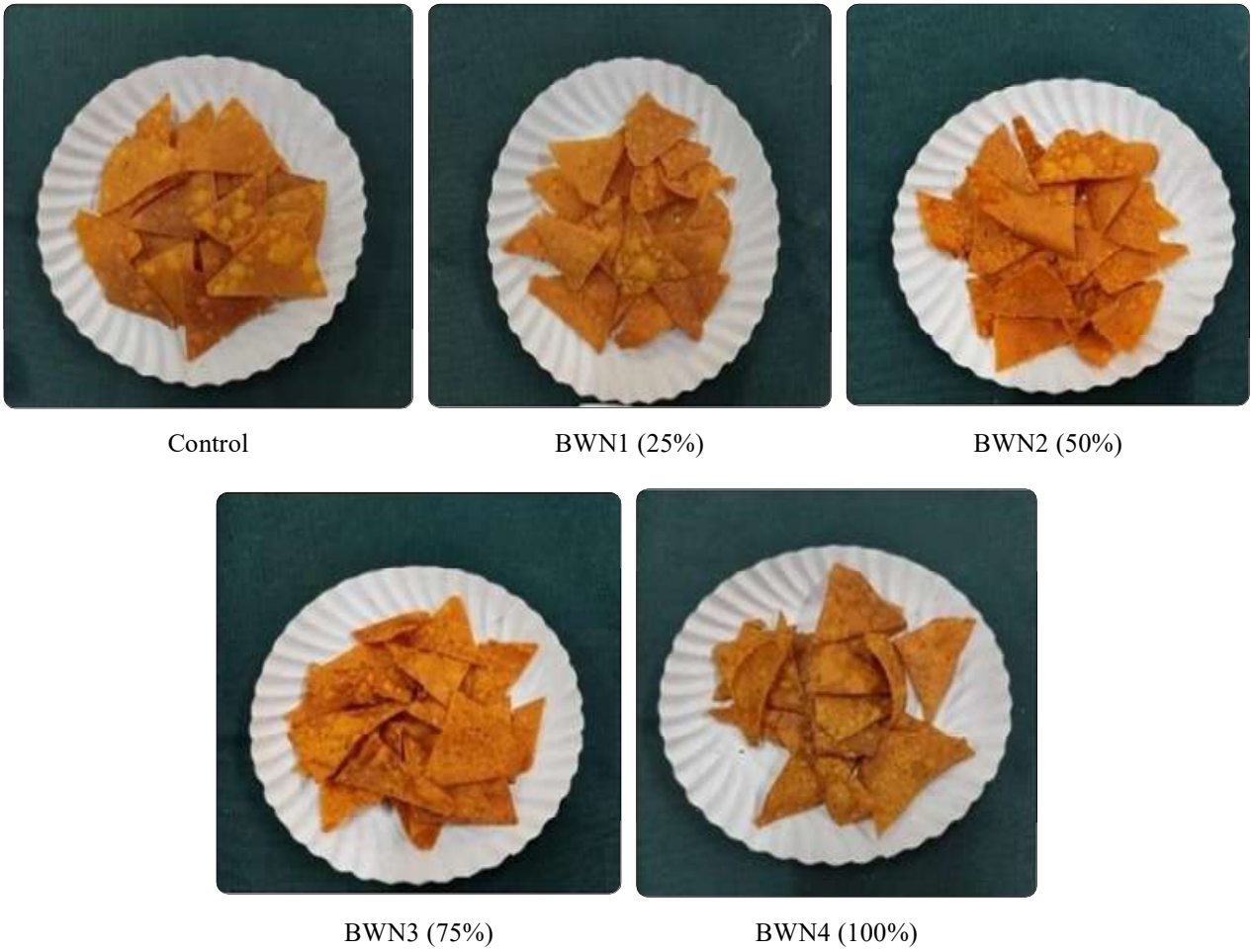

Plate 1 : Buckwheat *nachos* variations

samples were analyzed for moisture, peroxide value, free fatty acids and sensory evaluation for 60 days at the interval of 15 days.

Statistical Analysis

All the analyses were performed in triplicate and data was analysed using EXCEL and OPStat software. Analysis of variance (ANOVA), Critical difference and F-test were used to analyze data. Data was analysed using one-way ANOVA and in CRD to determine LOS. One-way analysis of the variance was applied for sensory scores, microbial population

Results and Discussion

Nutrient Composition of Buckwheat Flour

Buckwheat was dehulled and processed in to flour and subjected to further study. The nutrient analysis was carried out. Macro nutrient composition of buckwheat is presented in Table 1, providing an

Table 1

Macro nutrient composition of Buckwheat (per 100 g)

Macronutrients	Values
Moisture (%)	11.31 ± 0.08
Protein (g)	13.18 ± 0.35
Fat (g)	1.91 ± 0.10
Ash (g)	1.44 ± 0.05
Crude fiber (g)	2.20 ± 0.02
*Carbohydrate (g)	70.24 ± 0.33
**Energy (Kcal)	354 ± 1.03

Note : Values are mean ± standard
* difference method; **computation method

overview of its nutritional richness and potential health-promoting value. The moisture content of buckwheat was 11.31 ± 0.08 per cent, indicating moderate moisture, which helps in maintaining good storage stability and prevents microbial spoilage. The protein content was 13.18 ± 0.35 g/100g, suggesting that buckwheat flour is a good source of plant-based protein. The fat content was 1.91 ± 0.10 g/100g, which is relatively low and beneficial for developing low-fat food products. The ash content was 1.44 ± 0.05 g /100g shows the presence of essential minerals in the flour and contained 2.20 ± 0.02 g/100g of crude fiber. The carbohydrate content was 70.24 ± 0.33 g, making it a significant source of energy, while the total energy value was 354 ± 1.03 kcal/100g, suggesting that buck wheat flour can be effectively used in the preparation of various energy-rich food products. Similar findings were obtained by Devi *et al.* (2024) who reported that buckwheat flour had moisture (12.08%), ash (2.33%), fat (0.89%), protein (12.84%), carbohydrates (66.54%) energy content (325.53 Kcal/ 100g) and crude fiber (5.38 g/100g) respectively.

Micronutrient composition of buckwheat is depicted in Table 2. Potassium was found to be the most abundant mineral 419 ± 1.00 mg/100g playing a vital role in maintaining electrolyte balance, nerve transmission and muscle function. The buckwheat also had a significant amount of phosphorus (343.66 ± 1.52 mg/100g) and magnesium (225.33 ± 1.52 mg/100g), which are important for bone health, energy metabolism, and various enzymatic activities. The calcium content (38.25 ± 0.01 mg/100g) supports bone development and structural integrity. Trace minerals such as manganese (2.26 ± 0.02 mg/100g), copper (0.55 ± 0.02 mg/100g), iron (4.23 ± 0.04 mg/100g) and zinc (3.32 ± 0.02 mg/100g), though present in smaller amounts are vital for antioxidant defense, immune function and metabolic processes. The results of the study are in concurrent with the findings of Bhavsar et al. (2013) who reported that buckwheat flour had iron (2.67), zinc (2.53), manganese, (1.52) and copper (0.49) mg/100g respectively. Similar findings were obtained by Amarowicz and Fornal (1987) who reported that buckwheat flour contains potassium (299.4 mg/100g), magnesium (160.8 mg/100g), manganese (2.22 mg/100g), iron (5.26 mg/100g), zinc (3.72 mg/100g) and copper (0.53 mg/100g) respectively.

TABLE 2

Micro nutrient composition of Buckwheat flour (per 100 g)

Macronutrients	Values
Calcium	38.25 ± 0.01
Potassium	419.00 ± 1.00
Phosphorous	343.66 ± 1.52
Magnesium	225.33 ± 1.52
Manganese	2.26 ± 0.02
Copper	0.55 ± 0.02
Iron	4.23 ± 0.04
Zinc	3.32 ± 0.02

Note : Values are mean ± standard deviation

Sensory Evaluation of Developed Buckwheat *Nachos*

The sensory evaluation of *nachos* prepared from buckwheat flour incorporated with maize flour is depicted in Fig. 2. Among the treatments 100% buckwheat *nachos* (BWN4) received the highest mean scores for appearance (8.15), colour (8.16), texture (8.37), flavour (8.45), taste (8.26) and overall acceptability (8.43). The superior sensory scores indicate that complete substitution with buckwheat flour in *nachos* with an attractive brownish colour, appealing crispness and characteristic nutty flavour. On the other hand, the control *nachos* (prepared from maize flour) had notably lower scores in appearance (7.94), colour (7.86) texture (7.91), flavour (7.64), taste (7.72) and overall acceptability (7.63) respectively. Similar findings were reported by Patiballa and Ravindra (2022) who reported that 100 per cent replacement of corn flour with quinoa flour *nachos* had higher sensory scores of 8.52 (appearance), 8.93 (colour), 8.57 (flavour), 8.68 (taste), 8.76 (texture) and 8.89 (overall acceptability) respectively. Farooqui *et al.* (2023) reported that

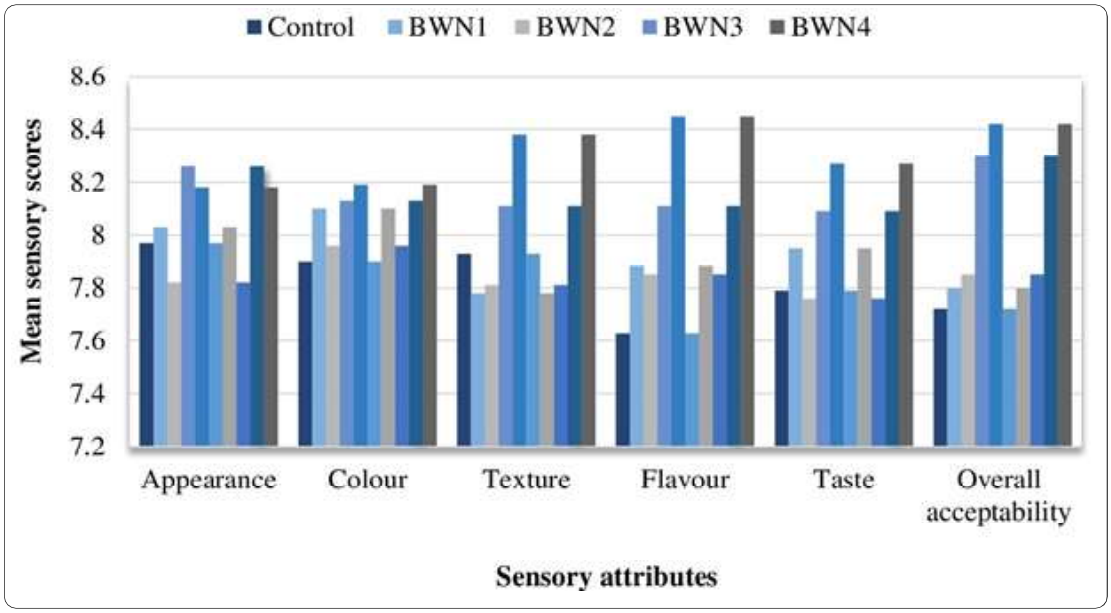

Fig. 2 : Mean sensory scores of buckwheat nachos

the 30% substitution of wheat flour with little millet flour (LMN3) *nachos* was most acceptable, recording high scores across sensory attributes, including appearance (8.17), colour (8.41), texture (8.29), flavour (8.00), taste (8.59) and overall acceptability (8.36) respectively.

Nutrient Composition, Phytonutrients and Total Antioxidant activity of Best accepted Buckwheat *Nachos*

Proximate composition of buckwheat *nachos* (BWN4) was analyzed and results are depicted in Table 3.

TABLE 3

Proximate composition of *nachos* (per 100g)

Macronutrients	Control	BWN4
Moisture (%)	4.26 ± 0.81	3.11 ± 0.01
Protein (g)	11.81 ± 0.76	13.99 ± 2.73
Fat (g)	18.33 ± 0.05	19.63 ± 0.07
Total ash (g)	1.73 ± 0.07	1.90 ± 0.30
Crude fiber (g)	2.46 ± 0.05	3.06 ± 0.15
*Carbohydrate (g)	61.39 ± 0.10	58.29 ± 2.40
**Energy (Kcal)	458 ± 3.75	466 ± 1.58

Note : Values are mean ± standard deviation * By difference ** By computation method Control - Maize flour *nachos*, BWN4 - Buckwheat *nachos* (100%)

Moisture content was found to be higher in the control *nachos* (4.26 g/100g) and lower in Buckwheat *nachos* (BWN4) (3.11 g/100g). Protein content was recorded to be higher in BWN4 (13.99 g/100g) compared to the control sample (11.81 g/100g). Similarly, fat content increased from 18.33 g/100g in the control to 19.63 g/100g in BWN4. Buckwheat *nachos* also provided a higher amount of ash and crude fiber (1.90 and 3.06 g/100g, respectively) compared to the control (1.73 and 2.46 g/100g). However, carbohydrate content was low in BWN4 (58.29 g/100g) when compared to the control *nachos* (61.39 g/100g). The energy value of buckwheat *nachos* was 466 Kcal/100g, slightly higher than the control (458 Kcal/100g). Similar observation was made by Patiballa and Ravindra (2022) who reported that quinoa-based *nachos* by substituting corn flour with quinoa flour showed that 100 per cent replacement of corn flour with quinoa flour had higher protein (13.12 g), fiber (8.83 g), and ash (3.92 g) contents, along with lower carbohydrate (50.41 g) and energy values (396 kcal/100 g). The results of the study are in concurrent with the findings Rachitha (2024) reported that grain amaranth based *nachos* by substituting maize flour with grain amaranth flour showed that 75 per cent

replacement of grain amaranth flour with maize flour had moisture (3.78 %), protein (11.24g), fat (19.25), total ash (2.79 g), crude fibre (6.36), carbohydrate (56.59) and energy was 431kcal respectively.

Micronutrient content of buckwheat *nachos* was analyzed and results are shown in Table 4. Results showed that buckwheat *nachos* (BWN4) contained significantly higher levels of minerals compared to the control. Calcium, phosphorus and potassium increased notably in BWN4 (87.58, 246.67 and 387.49 mg/100g) compared to the control (29.63, 165.55 and 288.92 mg/100g). Magnesium also improved from 93.10 mg in the control to 149.51 mg/100g in BWN4. Trace minerals such as iron, zinc, copper and manganese were higher in buckwheat *nachos,* with iron rising from 2.57 to 4.57 mg/100g and manganese increasing from 0.68 to 1.47 mg/100g. These results confirm that buckwheat is naturally rich in essential minerals and its incorporation greatly enhances the micronutrient profile of *nachos,* making buckwheat *nachos* a more nutritious snack option compared to the control.

Similar findings were observed by Rachitha (2024) who reported that grain amaranth based *nachos* by substituting maize flour with grain amaranth flour showed that 75 per cent replacement of grain amaranth flour with maize flour had Grain amaranth *nachos* (75% GA) with notably higher calcium (101.3 mg/100g) and iron (5.43 mg/100g) than the control. It also stands out for its magnesium (169.9 mg/100g) and manganese (2.44 mg/100g) levels.

Phytonutrients and total antioxidant activity of buckwheat *nachos* presented in Table 5 showed that phytonutrient content was higher in buckwheat *nachos* (BWN4) compared to the control sample. High tannin content of 0.53% (5.39 mg TAE/100g), phytic acid (314.63 mg/100g) and total antioxidant activity of 98.54% (270.48 mg AAE/100g) was observed in buckwheat *nachos* compared to control *nachos* (0.26% (2.65 mg TAE/100g, 215.62 mg/100g and 97.98% (268.92 mg AAE/100g respectively), indicating that inclusion of buck wheat enhanced the antioxidant potential of the product.

TABLE 4

Mineral composition of *nachos* (per 100g)

Macronutrient (g/100g)	Control	BWN4
Calcium (mg)	29.63 ± 0.89	87.58 ± 2.62
Phosphorus (mg)	165.55 ± 4.97	246.67 ± 7.40
Potassium (mg)	288.92 ± 8.67	387.49 ± 11.62
Magnesium (mg)	93.10 ± 2.79	149.51 ± 4.49
Iron (mg)	2.57 ± 0.08	4.57 ± 0.18
Zinc (mg)	1.69 ± 0.05	1.78 ± 0.03
Copper (mg)	0.19 ± 0.01	0.57 ± 0.02
Manganese (mg)	0.68 ± 0.02	1.47 ± 0.04

Note : Values are mean ± standard deviation Control- Maize flour *nachos*, BWN4- Buckwheat *nachos* (100%)

TABLE 5

Phytonutrients and total antioxidant activity of *nachos*

Products	Samples	Tannin		Phytic acid (mg/100g)	Total Antioxidant activity	
		(%)	mg TAE/100g		(%)	mg AAE /100g
Nachos	Control	0.26	2.65	215.62	97.98	268.92
	BWN4 (100%)	0.53	5.39	314.63	98.54	270.48

Note : Control- Maize flour *nachos,* BWN4- Buckwheat *nachos* (100%)

Texture Analysis of Best Accepted Buckwheat *Nachos*

Texture analysis of best accepted buckwheat *Nachos* depicted Table 6, *s*howed that the hardness of buckwheat *nachos* (BWN4) was lower than that of the control sample. The control nachos recorded a hardness value of 13.66 ± 2.41 N, whereas BWN4 showed a reduced hardness of 7.84 ± 1.45 N. This decrease in hardness may be due to the absence of gluten in buckwheat flour, which results in a less compact structure and a lighter, more breakable texture. Lower hardness values have also been reported in gluten-free or pseudo cereal-based snacks, where the unique starch and fiber composition produces a more tender and crisp product. The present findings indicate that incorporation of buckwheat flour improves the crispiness and bite quality of *nachos,* making BWN4 more acceptable in terms of texture compared to the control. Similar findings were observed by Bashir *et al.* (2025) who reported that incorporating watermelon rind powder significantly reduced the hardness of baked nachos. The control sample prepared with 100% corn flour (T0) exhibited the highest hardness value (1051.7 ± 0.3%), whereas the sample containing 20% watermelon rind powder (T2) showed the lowest hardness (286.6 ± 0.1%). The reduction in hardness

Table 6

Texture analysis of nachos

Macronutrient (g/100g)		Values
Nachos	Control	13.66 ± 2.41
	BWN4 (100%)	7.84 ± 1.45

Note : Control- Maize flour nachos, BWN4- Buckwheat nachos (100%)

Table 7

Effect of storage on sensory attributes of best accepted buckwheat *nachos*

Products	Duration (Days)	Sensory parameters					
		Appearance	Colour	Texture	Flavour	Taste	Over all acceptability
Control	Initial	8.33 ± 0.65	7.52 ± 0.51	7.85 ± 0.47	8.00 ± 0.44	8.09 ± 0.53	8.09 ± 0.30
	15	7.76 ± 0.62	7.14 ± 0.47	7.33 ± 0.48	7.61 ± 0.49	7.38 ± 0.49	7.19 ± 0.15
	30	7.61 ± 0.74	7.04 ± 0.38	7.04 ± 0.21	7.04 ± 0.21	7.28 ± 0.46	7.09 ± 0.30
	45	7.42 ± 0.50	6.66 ± 0.48	6.76 ± 0.62	6.09 ± 0.30	6.04 ± 0.21	6.14 ± 0.21
	60	7.04 ± 0.490	6.95 ± 0.38	6.52 ± 0.51	6.02 ± 0.53	6.90 ± 0.70	6.66 ± 0.70
	F value	*	*	*	*	*	*
	SE m ±	0.13	0.09	0.10	0.08	0.11	0.08
	CD	0.37	0.27	0.29	0.24	0.31	0.23
BWN4 (100%)	Initial	8.23 ± 0.43	8.04 ± 0.49	8.61 ± 0.49	8.42 ± 0.50	8.66 ± 0.48	8.66 ± 0.48
	15	8.00 ± 0.21	7.85 ± 0.57	8.00 ± 0.31	8.00 ± 0.31	8.09 ± 0.43	8.47 ± 0.51
	30	8.09 ± 0.30	7.81 ± 0.40	7.57 ± 0.50	7.85 ± 0.47	7.66 ± 0.48	7.81 ± 0.40
	45	7.33 ± 0.48	7.04 ± 0.21	6.95 ± 0.66	7.09 ± 0.30	6.66 ± 0.48	7.14 ± 0.47
	60	7.28 ± 0.46	7.00 ± 0.31	6.81 ± 0.60	6.76 ± 0.43	6.61 ± 0.49	6.71 ± 0.56
	F value	*	*	*	*	*	*
	SE m ±	0.08	0.09	0.11	0.09	0.10	0.10
	CD	0.23	0.26	0.32	0.25	0.29	0.30

Note : Values are mean ± standard deviation, *Significant at 5%, Control- Maize flour *nachos,* BWN4- Buckwheat *nachos*

with increased watermelon rind powder content can be attributed to its fiber's ability to retain moisture and disrupt the rigid structure of the flour matrix.

Storage Study of Buckwheat *Nachos*

Mean Sensory Scores of Buckwheat *Nachos* on Storage

Storage study of buckwheat *nachos* is presented in Table 7. During storage, all sensory attributes decreased significantly in both the control and buckwheat *nachos* (BWN4) with increased storage days. The control sample initially recorded scores of 8.33 for appearance, 7.52 for colour, 7.85 for texture, 8.00 for flavour, 8.09 for taste and 8.09 for overall acceptability and by the end of 60th day, these values declined to 7.04, 6.95, 6.52, 6.02, 6.90 and 6.66 respectively. Similarly, buckwheat *nachos* (BWN4) showed an initial high acceptability with scores of 8.23 for appearance, 8.04 for colour, 8.61 for texture, 8.42 for flavour, 8.66 for taste and 8.66 for overall acceptability. However, by the 60th day of storage, these scores decreased significantly to 7.28, 7.00, 6.81, 6.76, 6.61 and 6.71 respectively, indicating a significant ($p \leq 0.05$) decline across all attributes. The reduction in sensory scores during storage may be attributed to changes in flavour and texture caused by the gradual development of rancidity and loss of crispness due to moisture absorption and oxidation of fats. In a similar study conducted by Tiwari *et al.* (2011) on quality characteristic and shelf life studies of deep-fried snack prepared from broken rice and legumes by-product. The results indicated that sensory score for flavor, taste, and overall acceptability (OAA) was significantly reduced after 30 days. Rancid flavor was reported in all products after 30 days of storage. Similar observation was made by Patiballa and Ravindra (2022) who reported that mean sensory scores of *nachos* stored at ambient condition, a significant difference was found among two variations. As the storage period increased gradual decrease in the sensory scores was observed from initial to the 90th day. Control *nachos* had mean sensory scores of 5.76, 5.98, 5.32 and 6.32 for flavour, taste, texture and overall acceptability respectively. Conversely, quinoa *nachos* had scores of 6.78 (flavour), 6.46 (taste), 6.32 (texture) and 7.12 (overall acceptability) after 90 days of storage.

Effect of Storage on Moisture, Free Fatty Acids and Peroxide Value of Buckwheat *Nachos*

The data presented in Table 8 showed the effect of storage on the moisture content, peroxide value and free fatty acid (FFA) levels of the best accepted buckwheat *nachos* (BWN4) and control samples during storage period of 60 days at ambient condition. The results were significant at the 5% level. The

Table 8

Effect of storage on moisture, peroxide value and free fatty acid content of best accepted buckwheat *nachos*

Variation	Parameters	Duration (Days)					F value	SEm.±	CD
		Initial	15	30	45	60			
Control	Moisture (%)	4.28	5.44	6.20	6.91	7.89	*	0.01	0.04
	Peroxide value (meq O_2/kg of fat)	0.57	1.18	1.62	2.39	2.82	*	0.02	0.08
	Free fatty acid (mg KOH/kg)	0.16	0.25	0.35	0.56	0.65	*	0.01	0.06
BWN4 (100%)	Moisture (%)	3.09	4.36	5.05	5.79	6.45	*	0.01	0.03
	Peroxide value (meq O_2/kg of fat)	0.55	0.94	1.22	1.80	2.36	*	0.02	0.08
	Free fatty acid (mg KOH/kg)	0.13	0.21	0.32	0.44	0.61	*	0.01	0.03

moisture content of both control and buckwheat *nachos* increased progressively with the storage period. In the control sample, moisture content increased significantly from 4.28% initially to 7.89% on the 60th day, while in buckwheat *nachos* (BWN4), it rose from 3.09% to 6.45%. The peroxide value, which is an indicator of lipid oxidation and product rancidity, also increased significantly throughout the storage period. In the control, peroxide value increased from 0.57 to 2.82 meq O_2, /kg, while in buckwheat *nachos* it rose from 0.55 to 2.36 meq O_2, /kg by the end of 60th day these changes are statistically significant. Similarly, the free fatty acid (FFA) content increased gradually during storage. The control sample recorded an increase from 0.16 to 0.65 mg KOH/g, while buckwheat *nachos* showed a rise from 0.13 to 0.61 mg KOH/g. In similar study conducted by Patiballa and Ravindra (2022) reported that moisture content ranged between 4.08 and 9.06 for T1 (Corn nachos), while 3.06 to 7.98 per cent for T2 (100% Quinoa nachos) from initial to 90 days of storage. Free fatty acid was found to increase with storage period for T1 (0.18 to 0.92) and T2 (0.14 to 0.81) and peroxide value (PV) of the products increased from 0, 30, 60 and 90 days of storage for T1 (0.56, 1.62, 2.73 and 3.04) and T2 (0.50, 1.21, 2.26 and 3.02) samples, respectively.

The advocacy of buckwheat in India is not widely spread all over the country. Many of the people are unware of this crop and its being underutilized, inspite of its rich nutritional quality and health benefits. Buckwheat is a good source of protein, fibre, minerals and it is also rich in phytonutrients. Buckwheat *nachos* was acceptable at 100 per cent and the product exhibited superior nutritional composition, higher antioxidant activity and good textural characteristics. Buckwheat *nachos* was safe for consumption for 60 days when packed in MPP covers stored at ambient temperature. Thus, buck wheat can be used effectively in preparation of traditional snacks to promote the development of nutritious and healthy functional foods.

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Molecular Studies on Prospecting Antimicrobial Peptides from the Leaf Weaver Ant (*Oecophylla smaragdina*)

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Abstract

Antimicrobial peptides (AMPs) are crucial innate defense molecules with potential applications in agriculture and medicine. In this study, whole-body extracts of the leaf-weaver ant *Oecophylla smaragdina* were investigated for antimicrobial activity. Crude extracts were fractionated using preparatory RP-HPLC into two peptide-enriched fractions and peptide presence was confirmed by MALDI-TOF mass spectrometry with mass ranges of 304.4–2511.3 Da (crude extract), 212.7–2892.5 Da (fraction 1) and 543.1–1912.7 Da (fraction 2). Protein concentrations were 3.58 mg/mL for crude extract, 1.48 mg/mL for fraction 1 and 1.65 mg/mL for fraction 2. Antimicrobial activity was tested against *Xanthomonas oryzae*, *Escherichia coli*, *Staphylococcus aureus* and the phytopathogenic fungus *Alternaria alternata*. Crude extracts produced inhibition zones of 172.9 ± 34.6 mm² (*X. oryzae*), 141.5 ± 17.3 mm² (*E. coli*) and 84.8 ± 9.4 mm² (*S. aureus*). Fraction 2 at 100 µg/µL generated inhibition zones comparable to tetracycline against *X. oryzae* (333.9 ± 23.0 mm² vs. 430.1 ± 34.8 mm²). Both fractions significantly inhibited fungal mycelial growth with crude extract showing superior inhibition at early incubation stages. These findings confirm that *O. smaragdina* produces bioactive peptides with possible antibacterial and antifungal activity, underscoring its potential as a novel source of AMPs for sustainable crop protection and antimicrobial development.

Keywords : *Oecophylla smaragdina*, Antimicrobial peptides, Antifungal activity, Antibacterial activity

Antimicrobial peptides (AMPs) are short sequences of amino acids that play a critical role in innate defense across diverse taxa, including insects, vertebrates, plants, crustaceans, and microorganisms (Peterson and Kaur, 2018; Wu *et al.,* 2018). They are of growing interest as alternatives to conventional antibiotics because of their broad-spectrum activity and lower tendency for resistance development (Keerthi *et al.,* 2025; Sahu and Brahmaprakash, 2018; Merrifield *et al.,* 2007; Wang, 2014). Thousands of AMPs have been characterized from a wide range of organisms and their applications extend to medicine, agriculture and food preservation (Cammue *et al.,* 1992; Tam *et al.*, 2015; Santos *et al.*, 2018). Despite this diversity, AMPs from ant venoms remain relatively understudied compared to those from snakes, spiders, and scorpions (Radis *et al.,* 2015; Fratini *et al.,* 2017).

Ants (Hymenoptera: Formicidae) are ecologically dominant insects with more than 17,000 described species (Parr and Bishop, 2022). Their success in evolution has been partly attributed to their reliance on chemical defenses, including venoms and secretions that protect colonies from pathogens and predators (Cerda and Dejean, 2011). Ant venoms contain a variety of compounds, but short linear peptides are especially prominent in antimicrobial activity (Touchard *et al.,* 2015). Although studies have identified peptides with antibacterial, antifungal and

insecticidal properties in certain ant species (Aili *et al.,* 2014 and Touchard *et al.,* 2016), the diversity of ant-derived AMPs remains largely unexplored.

The leaf-weaver ant *Oecophylla smaragdina* is a widely distributed arboreal species in Southeast Asia and northern Australia (Bluthgen and Fiedler, 2002). Known for constructing leaf nests using larval silk, this species is ecologically important as both a territorial predator and an effective biological control agent in tropical agriculture (Peng and Christian, 2004). While *O. smaragdina* has been thoroughly studied for its role in pest management, its venom and peptide repertoire remain poorly characterized. Given the increasing need for novel antimicrobials in both clinical and agricultural settings, exploring *O. smaragdina* as a source of AMPs could yield new possibilities for bioactive compounds with dual applications.

In the present study, we investigated the antimicrobial potential of crude extracts and peptide fractions derived from whole-body homogenates of *O. smaragdina*. Using RP-HPLC fractionation and MALDI-TOF mass spectrometry, we identified peptide-rich fractions and assessed their activity against bacterial and fungal pathogens of medical and agricultural importance. This study provides the first evidence that *O. smaragdina* harbors bioactive peptides with potent antimicrobial activity, expanding the repertoire of ant-derived AMPs and highlighting their potential applications in sustainable disease management.

Material and Methods

Collection of *O. smaragdina* and Crude Peptide Extraction

Worker ants (5-7 mm) of *O. smaragdina* were collected manually from approximately 60 nests located in the Botanical Garden of the University of Agricultural Sciences, GKVK, Bengaluru and the National Bureau of Agricultural Insect Resources, Bengaluru, India. Collections were made between February 15 and April 25, 2021, during peak foraging activity (06:30-10:30 h and 14:00-17:00 h). Ant specimens were identified and voucher samples were deposited at the Bioprospecting Referral Collection, School of Ecology and Conservation, UAS, Bangalore.

Approximately 20,000 ants were pooled and processed for extraction. Ants were weighed in batches of 10 g, homogenized in acetonitrile: water (50:50, v/v) containing 0.1 per cent Tri Fluoro Acetic acid (TFA) and stored at -20 °C. Homogenates were subjected to ultrasonic disruption (pulse mode, 5 min), followed by centrifugation at 10,000 rpm for 10 min at 4 °C. Supernatants were concentrated using a vacuum concentrator at 30 °C, lyophilized and stored at $^{-}$80 °C until further use.

Protein Estimation

Protein content of crude extracts and fractions was determined with the Bradford assay, compared to Bovine Serum Albumin (BSA). Absorbance was measured at 595 nm using a microplate reader and protein concentrations were tested from the regression equation derived from the standard curve.

HPLC Fractionation

Crude extracts were fractionated by preparative reverse-phase High-Performance Liquid Chromatography (RP-HPLC) using a C18 column (Phenomenex, 5 µm, 250 × 4.6 mm) on a Shimadzu LC-20AT system equipped with a UV detector (λ = 214 nm). The binary gradient system consisted of solvent A (water with 0.1% TFA) and solvent B (acetonitrile with 0.1% TFA). Separation was achieved using the following gradient at flow rate of 5 mL/min: 0-1 min, 0-10% B; 1-16 min, 10-75% B; 16-19 min, 95% B; 19-22 min, 95-10% B. Manually collected fractions were lyophilized, stored at -20°C and divided into two principal fractions according to retention durations.

MALDI-TOF Mass Spectrometry

Peptide mass analysis of crude extracts and fractions was performed using a Bruker Ultraflex TOF/TOF mass spectrometer (Bruker Daltonics, Germany) equipped with a 337 nm nitrogen laser. Samples were

reconstituted in 0.1 per cent TFA, mixed with an equal volume of matrix solution (α-cyano-4-hydroxycinnamic acid), spotted onto a MALDI plate and air-dried. Spectra were acquired in both positive and negative ion modes across a mass range of m/z 200-3000 Da. Data were analyzed with Flex Analysis software (Bruker Daltonics, v2.0).

Microbial Strains and Culture Conditions

Test organisms included *Staphylococcus aureus* (MTCC 3160), *Escherichia coli* (MTCC 2692), *Xanthomonas oryzae* (MTCC 11102) and the saprophytic fungus *Alternaria alternata* (MTCC 3793), obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India.

Antibacterial Assay

Antibacterial activity was tested by the disc diffusion method. Bacterial cultures were adjusted to OD600 = 0.4 and mixed with molten nutrient agar before pouring into the sterile Petri plates. Sterile 6 mm paper discs (HiMedia, SD067) were impregnated with 10 µL of crude extract or fraction at concentrations ranging from 10-100 µg/µL (corresponding to 100-1000 µg per disc). Tetracycline (30 µg/disc) was used as a positive control, while solvent alone served as the negative control. Plates were kept and incubated at 37 °C for 12 h, and inhibition zones were measured in mm².

Antifungal Assay

Antifungal activity was evaluated against *A. alternata* using the poisoned food method. Extracts and fractions were incorporated into molten potato dextrose agar (PDA) at defined concentrations and poured into sterile Petri plates. Mycelial plugs (5 mm) from the actively growing cultures were placed at the center of each plate. Plates were incubated at 28 ± 2 °C for 8 days. Colony diameters were measured and percentage inhibition of mycelial growth was calculated relative to untreated controls following Satish *et al*. (2017).

Statistical Analysis

All the experiments were conducted in triplicate (three biological replicates with three replicates each). Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test in OPSTAT software. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Protein Estimation

The protein concentration of crude extracts and fractions of *O. smaragdina* was quantified by the Bradford assay. Crude extract contained 3.58 mg/mL protein, whereas fraction 1 and fraction 2 contained 1.48 mg/mL and 1.65 mg/mL, respectively (Table 1). This indicates that fractionation reduced total protein content, possibly due to the separation of specific peptide components during purification.

Table 1

Protein concentration of crude extract and peptide fractions of *O. smaragdina*was determined by Bradford assay

Sample	Protein concentration (mg/mL)
Crude extract	3.58
Fraction 1	1.48
Fraction 2	1.65

Antibacterial Activity of Crude Extract

Disk diffusion assays demonstrated that crude extracts inhibited the growth of all three tested bacterial pathogens. Inhibition zones were 172.9 ± 34.6 mm² for *X. oryzae*, 141.5 ± 17.3 mm² for *E. coli* and 84.8 ± 9.4 mm² for *S. aureus*. No inhibition was observed in solvent controls, confirming that activity was due to extract components. Positive control tetracycline disks produced zones of 391.1 ± 72.6 mm² (*X. oryzae*), 301.2 ± 28.1 mm² (*E. coli*) and 301.7 ± 1.0 mm² (*S. aureus*) (Fig. 1 and Table 2). The crude extract of *O. smaragdina* exhibited significant antibacterial activity against all tested pathogens with the highest inhibition observed against *X. oryzae,* followed by *E. coli* and *S. aureus*. Although the inhibition zones were lower than those of the tetracycline control, the results confirm the broad-spectrum antibacterial potential of the crude extract.

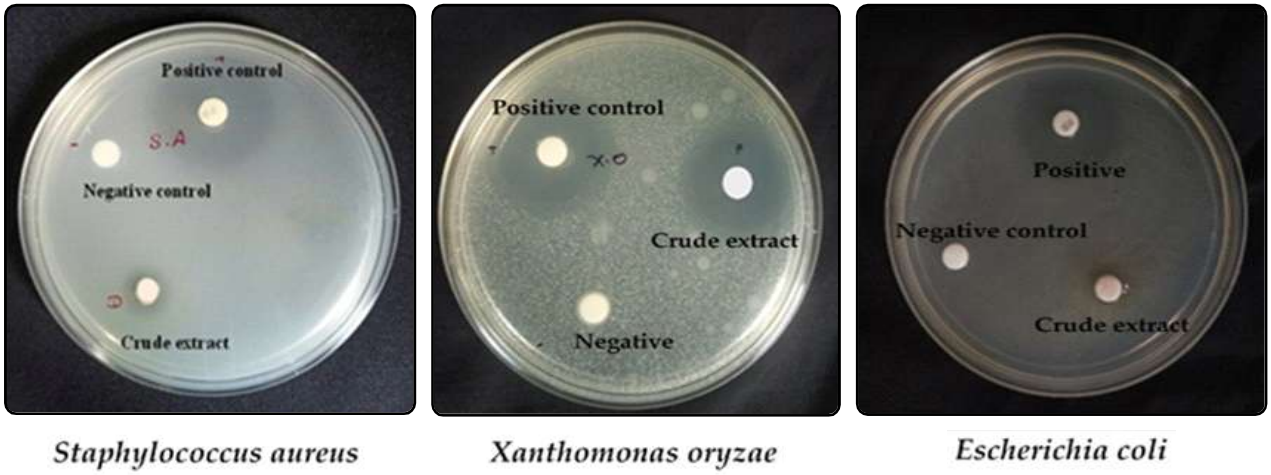

Fig. 1 : Antibacterial activity of crude extracts of *O. smaragdina*

TABLE 2

Antibacterial activity of crude extracts of *O. smaragdina* against selected pathogens expressed as inhibition annule (mm²) ± SD

Microorganism	Positive control (Tetracycline)	Crude extract	Negative control
X. oryzae	391.06 ± 72.63 [a]	172.94 ± 34.57 [c]	0
E. coli	301.21 ± 28.09 [b]	141.54 ± 17.26 [cd]	0
S. aureus	301.71 ± 1.00 [b]	84.75 ± 9.38 [d]	0

Values are mean ± standard deviation (n = 3). Different superscript letters within a row indicate significant differences at $p < 0.05$

HPLC Fractionation and Peptide Profiling

Preparative RP-HPLC resolved crude extracts into two major fractions based on retention times: fraction 1 (2.6–5.1 min) and fraction 2 (9.8–14.0 min) (Fig. 2). MALDI-TOF analysis confirmed the presence of peptides in crude extracts and fractions. The crude extract exhibited peptide masses ranging from 565 to 2511.3 Da (Fig. 3), fraction 1 from 313 to 1867 Da (Fig. 4) and fraction 2 from 909 to 1904 Da (Fig. 5). RP-HPLC successfully separated the crude extract of *O. smaragdina* into two distinct peptide fractions based on retention time, indicating compositional diversity in peptide polarity. MALDI-TOF MS profiling further confirmed the presence of low-molecular-weight peptides across all samples, suggesting that both fractions retained bioactive peptide constituents responsible for antimicrobial activity.

Antibacterial Activity of Fractions

Fractions displayed concentration-dependent antibacterial activity (Table 2). Fraction 1 inhibited *X. oryzae* with zones increasing from 15.9 ± 2.7 mm² at 10 µg/µL to 82.7 ± 8.0 mm² at 100 µg/µL. Against *E. coli* and *S. aureus*, inhibition was lower, reaching 15.8 ± 2.8 mm² and 37.7 ± 3.4 mm² at 100 µg/µL, respectively. Fraction 2 exhibited stronger activity with inhibition zones of 65.9 ± 5.4 mm² at 10 µg/µL and 333.9 ± 23.0 mm² at 100 µg/µL against *X. oryzae*. At 100 µg/µL, *E. coli* inhibition was 184.7 ± 12.4 mm², while *S. aureus* showed weak inhibition (6.9 ± 0.5 mm²). At the highest concentration, fraction 2 showed inhibition comparable to tetracycline for *X. oryzae* (333.9 vs. 430.1 mm²). Both peptide fractions of *O. smaragdina* exhibited dose-dependent antibacterial activity with Fraction 2 showing markedly higher inhibition against *X. oryzae* and

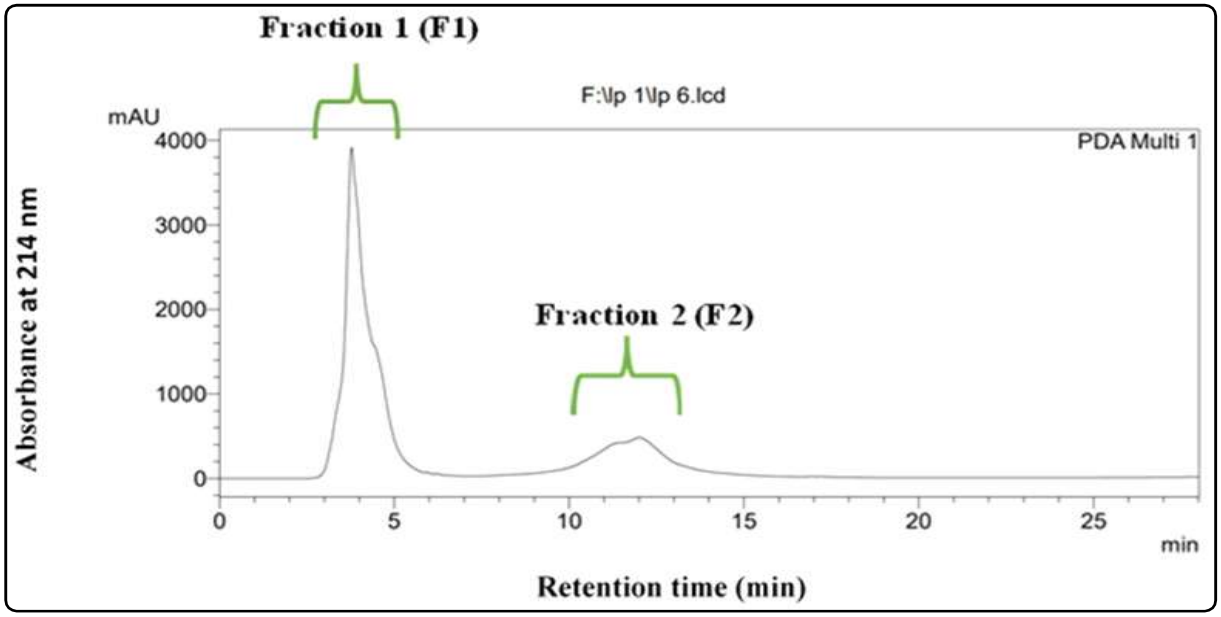

Fig. 2 : The RP-HPLC profile of crude extract from *O. smaragdina,* which has been subjected to Preparative Reverse-Phase HPLC; peptide fractions were selected manually based on retention time: fraction 1 (2.58-5.1min), fraction 2 (9.8-14 min)

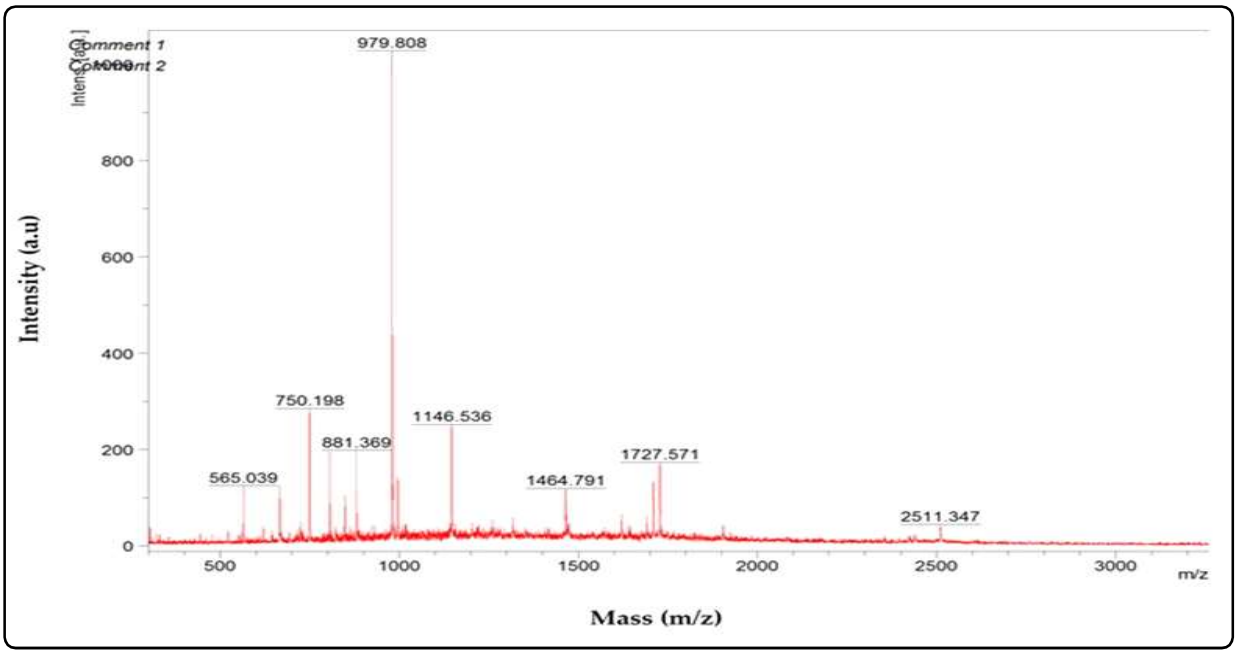

Fig. 3 : Peptide mass analysis of bioactive fraction of *O. smaragdina* by MALDI-TOF MS analysis using Ultraflex TOF/TOF mass spectrometer

E. coli compared to Fraction 1. However, neither fraction achieved inhibition comparable to the positive control, indicating moderate but selective antibacterial potency, particularly toward *X. oryzae*.

Antifungal Activity

Extracts and fractions inhibited *A. alternata* mycelial growth in PDA medium. Crude extract caused rapid

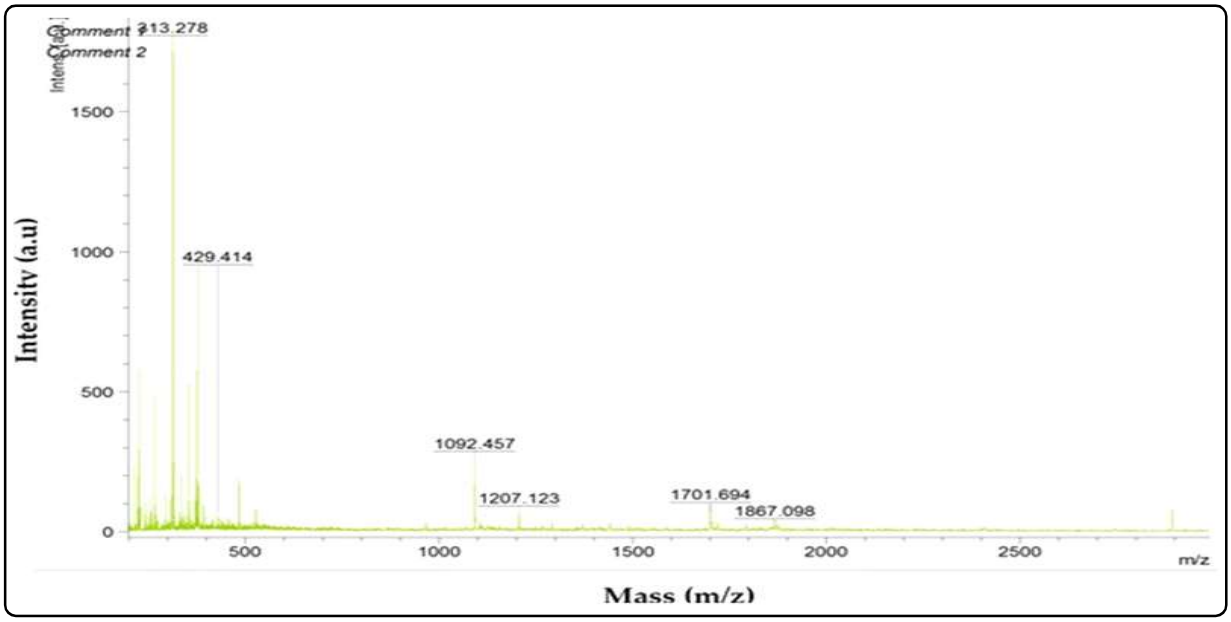

Fig. 4 : Peptide mass analysis of bioactive fraction 1 of *O. smaragdina* by MALDI-TOF MS analysis using Ultraflex TOF/TOF mass spectrometer

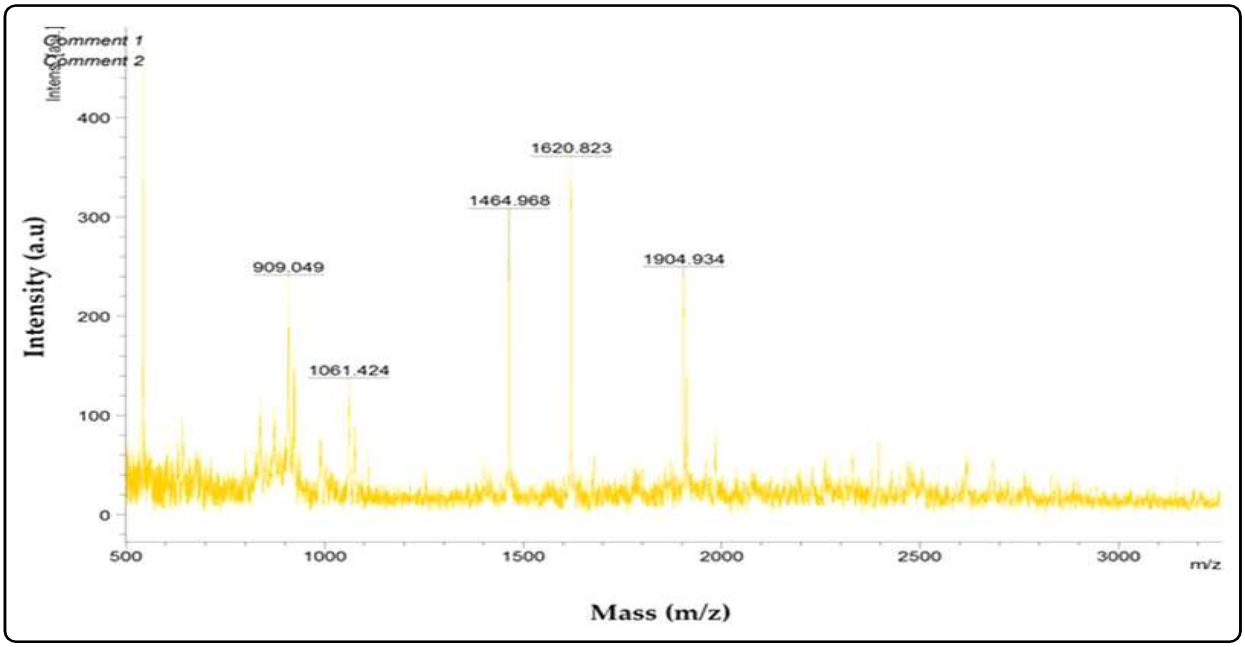

Fig. 5: Peptide mass analysis of bioactive fraction 2 of *O.smaragdina* by MALDI-TOF MS analysis using Ultraflex TOF/TOF mass spectrometer

inhibition, with significantly higher suppression at days 2–3 compared to both fractions. Fractions 1 and 2 displayed sustained inhibition throughout the 8-day incubation with no significant differences between them. Percent inhibition over control increased with time, confirming dose-dependent antifungal activity of both crude and fractionated peptides. the crude extract of *O. smaragdina* exhibited the highest antifungal activity against *A. alternata*, reaching a maximum inhibition of 50.51 ± 4.17 per cent on

TABLE 3

Antibacterial activity of peptide fractions of *O. smaragdina* against selected pathogens by Kirby-Bauer disc diffusion assay

Fraction	Level (µg/µL)	*Xanthomonas oryzae* (mm² ± SD)	*Escherichia coli* (mm² ± SD)	*Staphylococcus aureus* (mm² ± SD)
Fraction 1	NC	0	0	0
	10	15.88 ± 2.67 ^{h}	0	0
	50	65.99 ± 3.60 ^{h}	8.67 ± 0.56 ^{h}	17.52 ± 2.10 ^{h}
	100	82.67 ± 7.96 ^{ef}	15.81 ± 2.76 ^{h}	37.71 ± 3.37 ^{gh}
	PC	413.56 ± 16.14 ^{h}	327.41 ± 11.39 ^{bc}	301.24 ± 6.39 ^{c}
Fraction 2	NC	0	0	0
	10	65.99 ± 5.37 ^{fg}	0	0
	50	100.59 ± 3.12 ^{e}	63.02 ± 1.95 ^{fg}	0
	100	333.92 ± 23.01 ^{b}	184.66 ± 12.42 ^{d}	6.88 ± 0.47 ^{h}
	PC	430.06 ± 34.76 ^{a}	320.78 ± 23.89 ^{bc}	298.69 ± 3.66 ^{c}

Values are mean ± SD (n = 3). NC = Negative control; PC = Positive control (Tetracycline). Different superscript letters within a column indicate significant differences at $p < 0.05$

TABLE 4

Effect of crude extract and peptide fractions of *O. smaragdina* on mycelial growth inhibition of *A. alternata* over 9 days

No. of days	Crude (% inhibition ± SD)	Fraction 1 (% inhibition ± SD)	Fraction 2 (% inhibition ± SD)
1	24.79 ± 2.83 ^{bcdef}	19.85 ± 5.47 ^{cdefg}	5.99 ± 4.53 ^{g}
2	50.51 ± 4.17 ^{a}	15.31 ± 3.11 ^{efg}	22.10 ± 6.36 ^{bcdefg}
3	45.41 ± 15.08 ^{a}	13.37 ± 6.30 ^{fg}	15.11 ± 5.49 ^{efg}
4	33.11 ± 5.55 ^{abcde}	23.69 ± 4.33 ^{bcdefg}	23.58 ± 3.06 ^{bcdefg}
5	39.77 ± 7.71 ^{ab}	19.18 ± 7.01 ^{cdefg}	17.81 ± 5.90 ^{defg}
6	37.22 ± 6.26 ^{abc}	21.51 ± 3.62 ^{bcdefg}	16.18 ± 10.19 ^{efg}
7	35.94 ± 1.83 ^{abcd}	19.77 ± 1.78 ^{edefg}	16.14 ± 4.38 ^{efg}
8	35.25 ± 5.21 ^{abcd}	21.20 ± 5.31 ^{bcdefg}	17.70 ± 6.46 ^{defg}
9	33.38 ± 3.33 ^{abcde}	24.91 ± 2.41 ^{bcdef}	19.03 ± 0.67 ^{cdefg}

Values are mean ± SD (n = 3). Different superscript letters within a column indicate statistically significant differences at $p < 0.05$

day 2, followed by a gradual decline over time. In contrast, Fraction 1 and Fraction 2 showed lower inhibition levels (ranging from 13-25 and 6-24%, respectively) but maintained relatively stable activity throughout the 9-day incubation. This suggests that the crude extract possesses stronger initial antifungal compounds, while the peptide fractions exert prolonged but weaker inhibitory effects on fungal growth (Table 3).

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Performance Evaluation of Hand Operated Weeders for Dryland Agriculture

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Abstract

Weeding is an essential aspect of agriculture. Weeds are managed with the aid of herbicides. Hence the weeds are removed by mechanical methods. At present most of the farmers practicing traditional method of manual weeding, especially in smaller farming is combined with numerous problems they are time-consuming, less efficient, requiring significant human effort, which lead to physical strain and discomfort for laborers. Hence, experiment was conducted to study the performance of various advanced hand tools at COAE, UAS, GKVK, Bengaluru. Trials were performed to review the performance of different weeders namely, improved hand weeder, cycle weeder, single wheel three tyne hoe, twin wheel shovel type hoe. Various soil parameters such as moisture determination, bulk density prior to test and parameters such as weed control efficiency, field capacity, plant damage, field efficiency, draft and power input, Efficiency index of weeder were considered during the test. The field capacity of 0.027, 0.0037, 0.038 and 0.038 ha/hr respectively observed for improved hand weeder, cycle weeder, single wheel three tyne hoe, twin wheel shovel type hoe. Average draft and power requirement of the weeders were found as 11.08, 7.65, 4.42, 5.40 kg and 0.07, 0.06, 0.05, 0.06 hp, respectively. It is observed that there was no plant damage while carrying out the weeding with the above weeders.

Keywords : Manual weeder, Draft, Performance index, Weeding efficiency, Field efficiency

Agriculture plays a important role in the Indian economy, contributing about 15-16 per cent to the GDP and known for wide range of crops due to its varied agro-climatic conditions. Essential crops include paddy, wheat, maize, millets, pulses, cotton, sugarcane, oilseeds and various seasonal horticultural crops. It is the main source of income for a vast population, particularly in rural areas. As the population continues to grow, there is a pressing need to increase food and fiber production to meet rising demand. This can accomplished through either expanding the cultivated land or implementing modern farming technologies to boost crop yields (Singh *et al.,* 2020). Enhancing crop yield is a multifaceted endeavor that relies on multiple strategies and also the use of high-yield variety seeds, agronomic practices and effective measures to prevent yield loss caused by various factors, including weeds, insects, pests and diseases. Among these factors, weeds stand out as most significant contributors to crop yield loss (Shekhar *et al., 2010)*. To control weeds, herbicides must be used repeatedly. This repeated application of

herbicides leads to increase in environmental hazard and harms non-target organisms and neighboring crops due to chemical drift (Bindu *et al.,* 2023). Reduced weed growth in the initial stages resulted in better utilization of light, nutrients and increased photosynthetic efficiency leading to taller plants (Keerthana *et al.,* 2024). Weeds rival for essential resources like sunlight, water and nutrients. When not properly controlled, they can greatly reduce crop yields, disrupt harvesting, and sometimes result in total crop failure. Hence, weed management is a pivotal component of modern agriculture. The quality and quantity of crop yield depends upon effective and timeliness of weed removal from the field. Weeds causes highest annual yield loss of about 45 per cent compared to dieses (20%), insects (30%) and pests (5%) (Gupta *et al.,* 2014). Weeding involves one-third of cost of cultivation and accounts about 25 per cent of labour required during crop production (Kumar *et al.,* 1993 and Manjunatha *et al*., 2007). Removing weeds during the initial stages of crop growth is essential for optimizing crop yield. The initial 15 to 60 days following seed sowing represent a crucial period when weed development greatly reduce yield, resulting in a loss of approximately 16 to 42 percent. Weed removed by manually using tools like a khurpi, spade, hand hoe, demanding substantial labor input and involving labor-intensive and time-consuming work. Furthermore, the labor needed for weeding varies factors such as type of weeds present, their density, the timing of the weeding operation, soil moisture conditions and the efficiency of labour. (Sahu, M. and Goel, A. K., 2017). Timely weeding is the important farming operations for increased production and is a major determinant in effective weed control (Mohan, S. S., 2020). Weeds can be controlled by mechanical, chemical, biological and traditional methods. Among the different methods, weed control by mechanical method is the best with no limitation because of its effectiveness in keeping the soil tillage can mulch the soil, which results air circulation with in soil and water conservation through runoff (Hari *et al*., 2019 and Manjunatha *et al.,* 2014). As the entire State faces an acute shortage of labour, there is need for assessement of suitable weeding equipment is necessary. Manual labour requirement for weeding about 25% (900-1200 manhours/hectare) during a crop production (Yadav and Pund, 2007). Moisture of soil found to be 11.63 per cent optimum for weeding in groundnut and the operation cost of developed weeder at this moisture was found to be Rs.244.00 as against Rs.2450.00 per hectare in conventional method of weeding by using trench hoe (Mishra, *et al.,* 2008). Considering the socio-economic factors of farmers and the nature of their land holdings, a cost-effective and light weight manual weeder emerges as the most suitable option for mechanized weeding. In India, there is a wide variety of hand weeders available, each tailored to specific regions to accommodate the diverse needs related to soil types, crops, and cropping patterns (Alizadeh, M. Z. 2011). Therefore the experiment was undertaken to assess the efficiency and performance of weeders in real practical conditions.

Material and Methods

Constructional Details of Weeders

Improved Hand Weeder

The hand weeder consist of long handle with one number of 25 mm diameter and 1200 mm length pipe of which 520 mm long handle is fitted. At rear side of the middle pipe, two arms of size 250 X 25 X 3 mm of Mild steel flats are fitted. Star wheel is fixed at the end of the arm with 120 mm diameter. The star wheel facilitates the easy movement of tool and cutting blade is fitted to the arm of about 200 mm. The width of cut using blade is 120 mm.

Cycle Weeder

The manually operated push-type cycle weeder consists of a single spoked wheel fabricated from mild steel, having a diameter of 640 mm and width of 30-50 mm. Inverted 'V' type long handle, fixed with nut and bolt to wheel frame of length 1120 mm and diameter of 25 mm, positioned at a height of 1000 mm. A weeding blade of size 170 × 45 × 9.5 mm is mounted below the frame, just behind the wheel.

Single Wheel Three Tyne Hoe

Single-wheel three-tyne hoe main frame is fabricated from a mild steel pipe of length 1000 mm and 25 mm diameter. It is equipped with an inverted 'V' type handle, extended backward for operator comfort, with a handle height from ground level of 950 mm at a distance of 400 mm. A 550 mm diameter single rubber-tyred spoked wheel is mounted at the front. The blade assembly consists of three tynes fabricated of high carbon steel, each having dimensions of 180 × 75 × 10.5 mm.

Twin Wheel Shovel Type Hoe

Double circular wheels, each 300 mm in diameter, fabricated from mild steel. Fixed on the main frame made angle iron of size 175 × 120 × 12 mm. An adjustable solid mild steel pipe, 900 mm in length, is fitted between the wheels to enable the operator to push/pull and control the tool during operation. A shovel-type blade of size 25 × 45 × 12.5 mm is attached beside the two wheels for effective weeding between row to rows.

Experimental Procedure

The performance evaluation weeders was conducted in sandy loamy soil at Dry land agriculture, UAS, GKVK, Bengaluru. The distance between row to row was 65 cm and plant to plant was 45 cm. The crop was 30 days old and the field was infested with grass weeds and total of three replication were conducted. The density of weed at the time of weeding was 30 per m^2. The field was divided into 4 plots each of size 10 x 10 m in groundnut field. The experiment was carried for weeders in randomized design and three replications.

Fig. 1 : Before weeding

Fig. 2 : After weeding

Fig. 3 : Weeding Operation Using Single Wheel Three Tyne Hoe

Fig. 4 : Data collection

Moisture Content

Samples were collected in each plots with help of auger. The samples was kept in hot air oven at 105°C for 8 hours (Srinivas *et.al.,* 2010). Samples were kept for outside for cooling then weight of wach samples were noted down. The Moisture content on dry basis has been evaluated using the formula

$$\text{MC (\%)} = \frac{W_1 - W_2}{W_2} \times 100$$

Where,

W_1 - Weight of wet soil sample, kg

W_2 - Weight of the dried soil, kg.

Power

The input power required for weeding operation was calculated by considering draft and speed by using following equation (Hari *et al.,* 2019)

$$\text{Power (hp)} = \frac{D \times S}{75}$$

Where,

D = Draft, kg

S = Travelling speed, m/sec.

Weeding Efficiency

Number of weeds was counted before operation and number of weeds left after operation in a 1 m^2 area was recorded. Weeding efficiency was expressed by following formula (Tsegaye, A and Qabaradin, A., 2021).

$$\text{Wedding efficiency} = \frac{W_1 - W_2}{W_1} \times 100$$

Where,

W1 = No. of weeds before operation, per square meter.

W2 = No. number of weeds after operation, per square meter.

Plant Damage

Plant damage percentage was calculated by recording number of plants in 15 m row length before weeding and number of plant damaged in 15 m row length after weeding. Plant damage was evaluated by following formula (Veerangouda *et.al.,* 2010).

$$\text{Plant damage, (\%)} = (1 - (\frac{q}{p}) \times 100$$

Where,

q = No. of plants in a 15 m row after weeding

p = No. of plants in a 15 m row before weeding

Field Capacity

Actual area covered by the implement, based on total time utilized and width. Effective field capacity (ha/ h) is given by:

$$\text{EFC} = \frac{A}{T_P + T_I}$$

Where,

A = Area covered, ha

Tp = Productive time, h

Ti = Non-productive time, h

Field Efficiency

The percentage of time the machine operates at its full rated speed and width.

$$\text{FE (\%)} = \frac{\text{Actual field capacity}}{\text{Theoritical field capacity}}$$

Depth of weeding

The depth of weeding plays an important role for the draft of weeder and it was measured in the field.

Draft of weeder

Soil resistance during its forward movement at rated width and depth. (Hari *et.al.,* 2019)

$$D = W \times d_w \times R_s$$

Where,

D = Draft of weeder, kg

W = Width of cut, cm

d_w = Depth of cut, cm

R_s = Soil resistance, kg/cm^2.

Performance Index

The performance index of weeder found using the following equation.

$$PI = \frac{a \times q \times e}{P}$$

Where,

a = Field capacity, ha/h,

q = Plant damage factor, per cent.

e = Weeding index, per cent,

P = Power input, hp

Speed of Operation

The speed of operation for each weeders was measured in a test plots at distance of 10-meter between crop rows was marked in all the plots (Kumar *et.al.*, 2018). The time taken to cover 10 m of distance were recorded using a stopwatch. Total of four trails were conducted for each type of weeder in their respective field plots (Kumar *et.al.*, 2014). The crop parameters such as number of crop plants in the row, the number of damaged plants, and density of weeds before and after the operation, as well as the total field time and the actual time required for weeding with different types of weeders were recorded to evaluate the performance of the weeders.

Results and Discussion

The groundnut crop was approximately 30 days old when the weeding operation was carried out, with a row-to-row spacing of 400 mm. At the time of weeding, the soil moisture content averaged 10.2%. The plant population was about 20 plants per square meter and the mean plant height was recorded as 25 cm. The field experiments were performed in accordance with the RNAM and BIS testing standards and procedures, and the results obtained are presented in Table 1. The following treatments were considered for the field evaluation study.

T1 = Weeding by using improved hand weeder

T2 = Weeding by using sprocket cycle weeder

T3 = Weeding by using single wheel three tyne hoe

T4 = Weeding by using twin wheel shovel tyne hoe

Field Capacity

Single wheel three tyne (T3) and hoe twin wheel shovel tyne hoe (T4) weeders was found maximum effective field capacity of about 0.038 ha/h, which was higher compared to improved hand weeder (T1) and cycle weeder (T2) of 0.027 and 0.037 ha/h (Table 1). Due to its faster movement and its width it can cover larger field so that it has highest effective field efficiency compared to other weeders. Area coverage with wheel and hoe in wheat crop was 0.36 ha/day which was much lower than the T3 and T4 (Garg and Sharma 1998). The forward speed of T3 and T4 weeders was about 3.42 and 3.27 km/hr that of the T1 and T2 is 1.84 and 2.40 km/hr, hence the difference in effective field capacity.

Table 1

Weeding Efficiency

Particulars	Weeding depth, cm	Weeding efficiency %
Improved hand weeder	5.5	95.45
Sprocket cycle weeder	2.5	80.57
Single wheel three tyne hoe	2.5	91.33
Twin wheel shovel tyne hoe	2.5	83.20

Weeding Efficiency

Maximum weeding efficiency was observed with 'improved hand weeder T1 (95.45 %) followed by single wheel three tyne hoe 'T-3' (91.33 %), and by 'twin wheel shovel tyne hoe T4' (83.20 %) and sprocket cycle weeder 'T-2' (80.57 %). It is observed that, weeding efficiency depends on shape of blade, moisture content of soil at testing plot and cutting depth of weeder blade. The maximum weeding efficiency is obtained with improved hand weeder was observed because of the capability of this hand tools to work between plant to plant spaces in a row and maximum working depth of about 5.5 cm. Zero plant damage observed in improved hand weeder. It is concluded that weeder is more efficient because

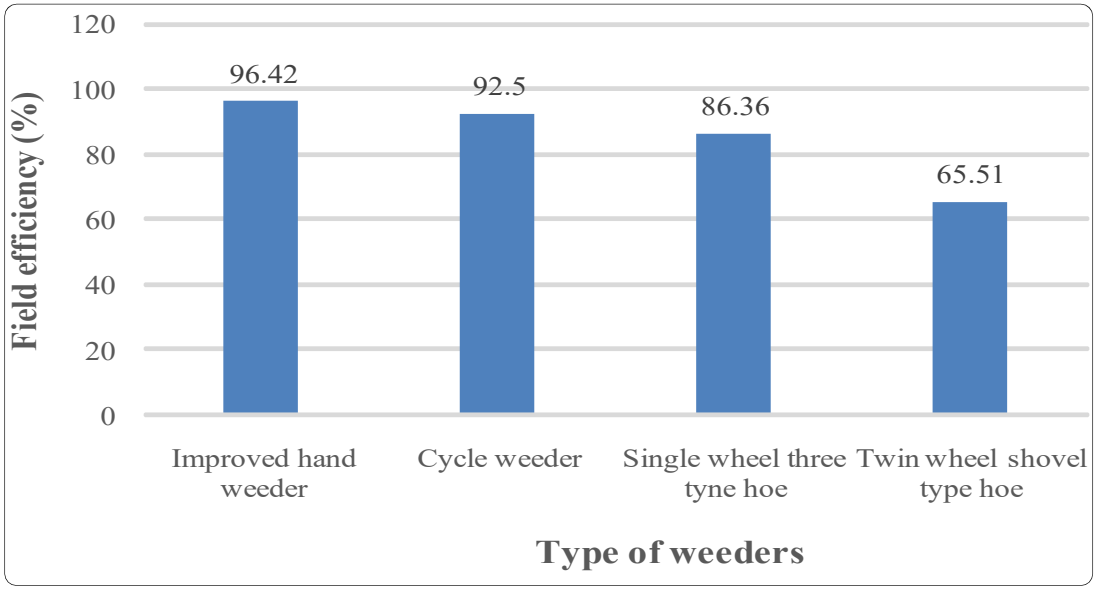

Fig. 5 : Effect of forward speed on Field Efficiency (%)

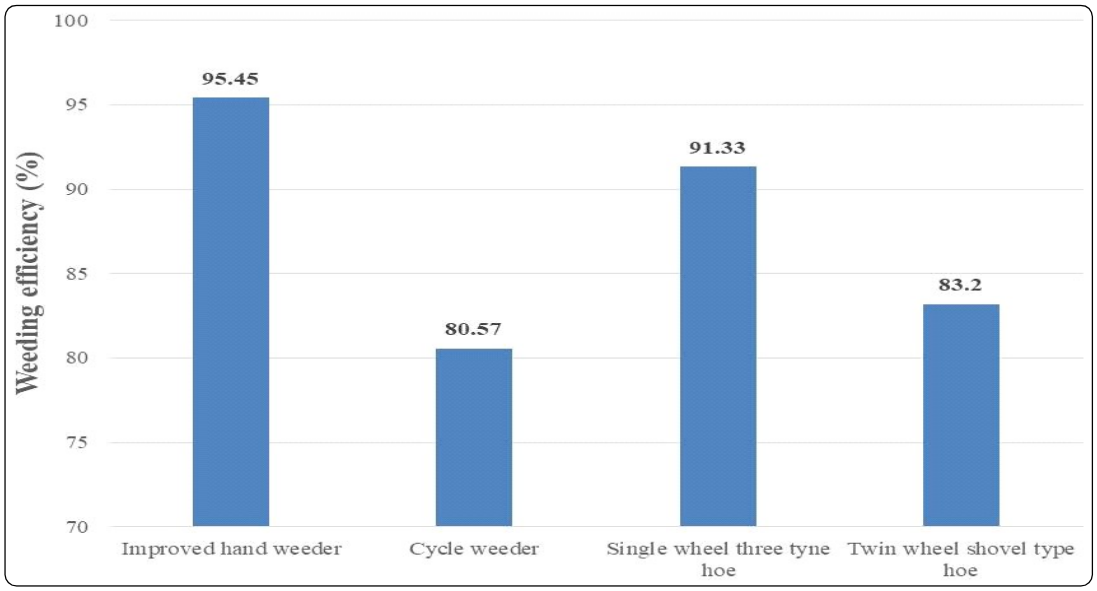

Fig. 6 : Effect of Machine Operational Parameters on Weeding Efficiency (%)

efficiency is more than 95 per cent and also more comfortable to work and less weeding efficiency was observed in sprocket cycle weeder due to less working depth of 2.5 cm.

The performance index, which depends on the weeding index, field capacity, power input and plant damage, was found to be highest (105.38) for the T4 weeder and lowest (36.81) for the T1 weeder. The overall field performance of the weeders is summarized in Table 2. The results indicated that the developed weeder is suitable not only for groundnut cultivation but also for other crops, since the row spacing can be adjusted accordingly. In addition, the penetration angle of the blades can be modified based on the operational requirement.

TABLE 2

Effect of working width on percentage of damage of ragi crop

Tool	Tool Effective working width, cm	Percentage of damage (%)
Improved hand weeder	15.5	0
Sprocket cycle weeder	17.00	2
Single wheel three tyne hoe	13.00	0
Twin wheel shovel tyne hoe	18.00	2

TABLE 3

Effect of speed of operation on efficiency

Tool	Speed of operation kmph	Field efficiency. %
Improved hand weeder	1.85	96.42
Sprocket cycle weeder	2.40	92.50
Single wheel three tyne hoe	3.42	86.36
Twin wheel shovel tyne hoe	3.27	65.51

The lowest performance index (36.8) was observed with the improved hand weeder (T1), mainly due to its manual operation, limited working width, and higher labor requirement. In contrast, the twin wheel shovel type hoe (T4) recorded the highest performance index (105.38) owing to its dual wheel support, efficient shovel blade design, and wider coverage, which enhanced stability, reduced drudgery, and improved field efficiency. The comparison indicates that improvements in design and operational characteristics have a direct impact on enhancing performance and Productivity Draft and power requirement are important parameters for a weeder, and they should remain within the physical capacity of the operator. The average draft values recorded for weeders T1, T2, T3, and T4 were 11.08, 7.65, 4.42, and 5.4 kg, respectively. The draft mainly depends on factors such as soil type, effective cutting width, and depth of cut. It was observed that weeder T1 required the highest draft, which may be attributed to its greater working width compared to the other weeders.

It was observed that the *Improved hand weeder* ($T_{1)}$ and *Single wheel three tyne* ($T_{3)}$ *hoe* recorded 0% plant damage, indicating that these implements are highly crop-friendly and do not adversely affect the standing plants during weeding operations. On the

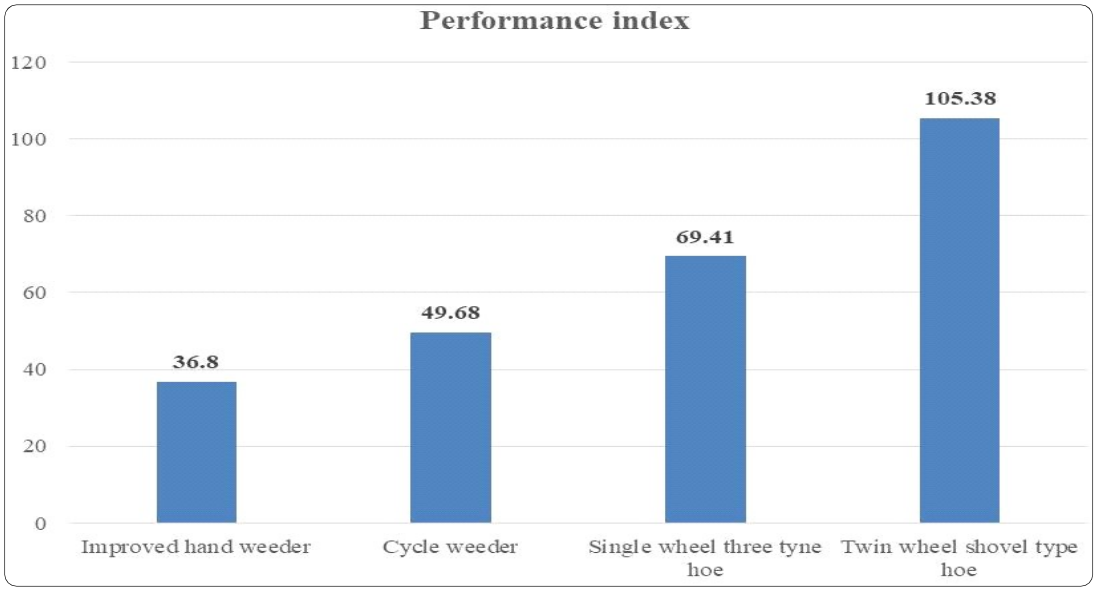

Fig. 7 : Effect of machine operational parameters on performance index

TABLE 4

Field performance of the manually operated weeder

Description	Improved hand weeder	Sprocket cycle weeder	Single wheel three tyne hoe	Twin wheel shovel tyne hoe
Soil moisture content, % (db)	12.5	11.5	11.5	11
Bulk density before operation, g/cc	1.38	1.40	1.40	1.41
Bulk density after operation, g/cc	1.35	1.37	1.38	1.38
Speed (km/hr)	1.85	2.4	3.42	3.27
Weeding efficiency, %	95.45	80.57	91.33	83.20
Plant damage, %	0	2	0	2
Effective working width, cm	15.5	17	13.0	18
Average working depth, cm	5.5	2.5	2.5	2.5
Draft requirement, kg	11.08	7.65	4.42	5.4
Effective field capacity, ha/h	0.027	0.0037	0.038	0.38
Field efficiency, %	96.42	92.50	86.36	65.51
Power (hp)	0.07	0.06	0.05	0.06
Performance index	36.81	49.68	69.41	105.38

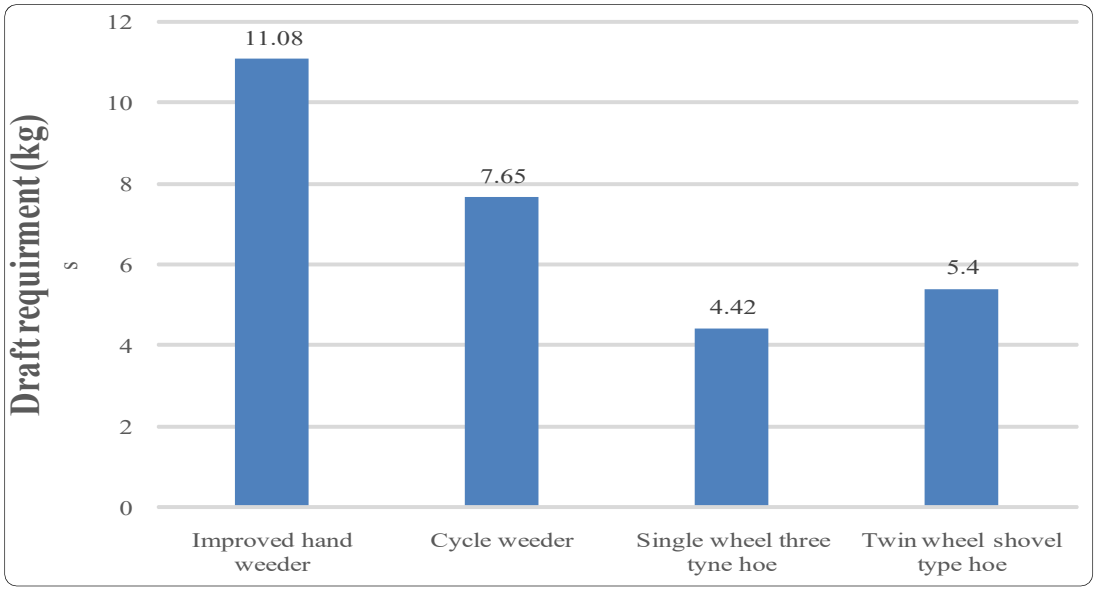

Fig. 8 : Effect of Machine Operational Parameters on Draft Requirement (kg)

other hand, the *Cycle weeder* (T_2) and *Twin wheel shovel type hoe* (T_4) showed a plant damage of 2 %, which may be attributed to the close proximity of their working components to the crop rows and their relatively higher operational aggressiveness. Although the level of plant damage caused by these tools is minor, it highlights the need for careful handling while operating such weeders in order to minimize crop loss. Overall, the findings suggest that the *improved hand weeder* and *Single wheel three tyne hoe* are safer options for reducing crop damage, whereas the *Cycle weeder* and *Twin*

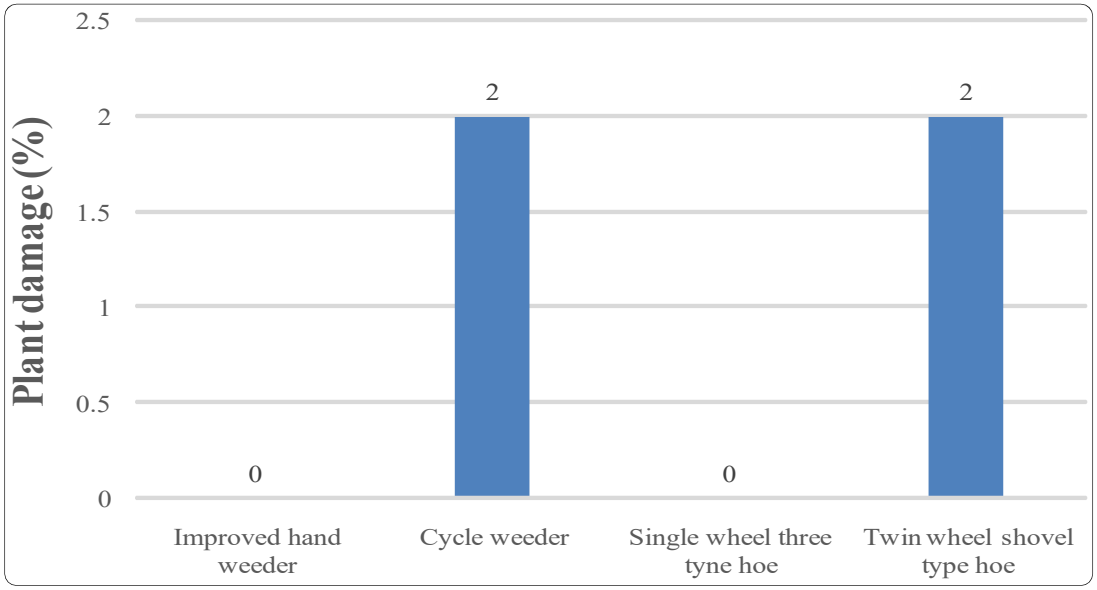

Fig. 9 : Effect of Machine Operational Parameters on Plant Damage (%)

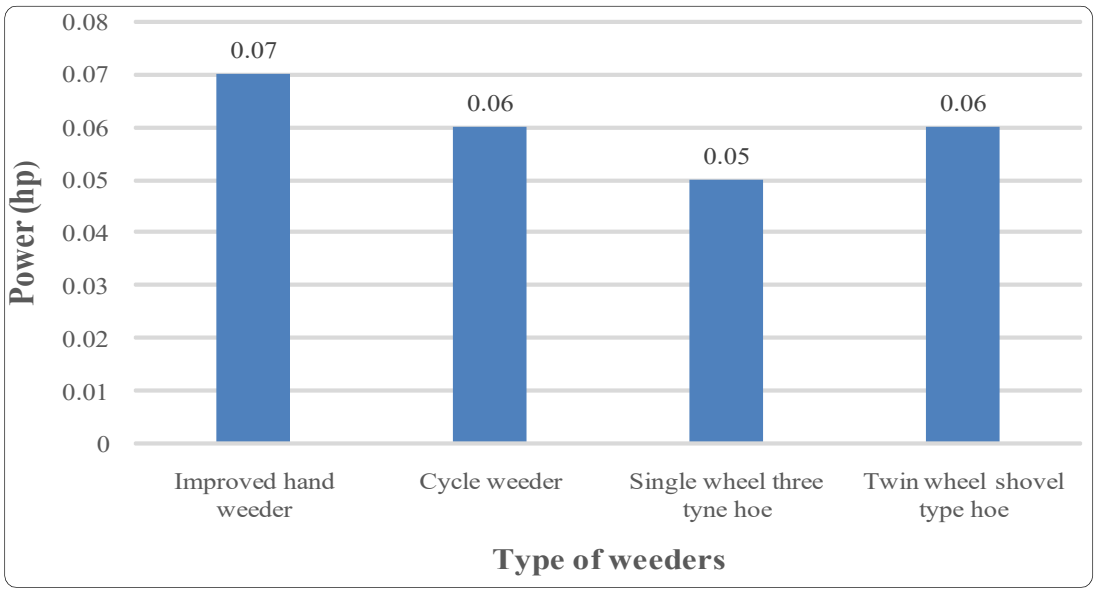

Fig. 10 : Effect of Draft on Power Requirement (hp)

wheel shovel type hoe require more precision in field operation.

The power requirement of improved hand weeder (T_1) recorded the maximum power requirement of 0.07 hp, followed by the Cycle weeder (T_2) and Twin wheel shovel type hoe (T_4), which required 0.06 hp each. The Single wheel three tyne hoe (T_2) exhibited the minimum power requirement of 0.05 hp. The variation in power requirement can be attributed to the design and operational characteristics of the weeders. The higher value observed for the improved hand weeder may be due to its greater soil-engaging surface and higher resistance during operation. In contrast, the single wheel three tyne hoe required the least power,

indicating better operational efficiency and reduced drudgery. The cycle weeder and twin wheel shovel type hoe showed intermediate power values, suggesting a moderate level of effort for effective weeding.

The performance of different weeding tools was evaluated and compared. The results showed that the improved hand weeder recorded the highest average weeding efficiency (95.45%) and an effective field capacity of 0.027 ha h^{-1}, with no plant damage, compared to the other weeders tested. In contrast, the single wheel three-tyne hoe weeder required the minimum power input and also resulted in zero plant damage (0%). It also achieved a higher average field capacity (0.038 ha h^{-1}, weeding efficiency (91.33%), (86.36%). and field efficiency Therefore, the improved hand weeder and the single wheel three-tyne hoe weeder were identified as the most effective and efficient tools for weeding operations.

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Value Chain Mapping and Price Spread of the Seaweed Sector in India

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Abstract

Seaweed farming has become a key source of income for many coastal families in Ramanathapuram district of Tamil Nadu, where the coastal environment is naturally well-suited for its cultivation. This study has closely examined at the seaweed value chain, drawing on primary data from 120 cultivators along with supporting insights from secondary sources. The results showed that farmers received an average farm-gate price of Rs.80 per kg, accounting for only a small share of the consumer price. Among the five marketing channels identified, channel III and channel IV were turned out to be more beneficial for farmers, each followed by 25 per cent of the farmers, with an average sale of 450 kg per month. channel I was the least preferred, adopted by only 10 per cent of farmers with an average sale of 250 kg per month, while channel V, the export-oriented route, recorded the moderate sales of 350 kg per month. The total marketing cost across channels ranged from Rs.304.90 to Rs.814.95 per kg and the price spread varied between Rs.590.82 and Rs.1,863.68 per kg. Farmers share in the consumer rupee ranged from 4.12 to 11.93 per cent, with the marketing efficiency index varying between 1.03 and 1.08, indicating that shorter channels provided better returns. The study also found that seaweed yield and income fluctuate with changes in climatic conditions, sea temperature and water quality. Constraint analysis highlighted low farm-gate prices (RBQ score 90.28), seasonal employment (81.25), poor protective gear (77.78) and price fluctuations (79.20) as the most pressing issues across the value chain. The study concludes that promoting collective marketing, improving drying and storage infrastructure and strengthening linkages with processors and retailers through cooperatives and digital platforms can improve farmer margins and enhance the overall efficiency of the seaweed sector in India.

Keywords : Seaweed, Value chain analysis, Price spread, Marketing efficiency, Consumer rupee

The seaweed has emerged as an economically and environmentally sustainable livelihood choice for many coastal communities in India. Particularly in Tamil Nadu, raft-based seaweed farming is gaining prominence as a reliable source of income, mainly for women, who comprise a significant proportion of cultivators. Unlike wild harvesting, which relies on seasonal availability and natural stocks, seaweed farming enables controlled and year-round production. The knowledge of cultivation is often passed down through local institutions, community networks and hands-on training, similar to traditional knowledge systems seen in coastal livelihoods globally. As with many other rural occupations, seaweed cultivation is deeply embedded in the socio-economic fabric of these communities, particularly in regions like the Gulf of Mannar and Palk Bay.

Seaweed, often termed the 'Wonder Plant of the Sea' holds immense commercial and ecological value. The global seaweed industry is projected to reach a market size of US $26 billion by 2025. India, home to

approximately 865 documented seaweed species including 442 red, 212 green and 211 brown varieties has shown growing interest in seaweed farming (CMFRI, 2024). Despite this diversity, the country's cultivated seaweed production remains modest, contributing only about 0.02 per cent of global seaweed cultivation (Cai *et al.,* 2021). India's cultivation efforts are presently centered around *Kappaphycus alvarezii*, the only variety of species that has been successfully domesticated and commercialized, forming the entire base of India's farmed seaweed output.

Unlike wild harvesting, which involves multiple naturally available species, cultivated seaweed farming offers scalability, predictability and opportunities for technological interventions. Organized studies and documentation of the cultivated seaweed value chain remain sparse. Examining this value chain is essential to optimize production and post-harvest processes, enhance income for cultivators and ensure equitable distribution of benefits. A detailed value chain analysis serves as a strategic tool to identify inefficiencies, reduce marketing costs and enhance overall productivity and competitiveness (Arjuman *et al.,* 2022).

This study aims to map the production-to-consumption system of raft-cultivated seaweed of *Kappaphycus alvarezii*, by identifying key actors and their functions along the value chain. It also analyzes variable costs, profit margins across stages and scale up the seaweed farming sector in India.

Material and Methods

Study Area

Seaweed cultivation in India is primarily concentrated along the southeast coast, with the Gulf of Mannar and Palk Bay in Tamil Nadu being the most prominent regions. These areas offer ideal environmental conditions such as shallow waters, moderate tidal flow and optimal salinity making them most suitable for raft-based seaweed farming. The Gulf of Mannar was designated a Marine Biosphere Reserve under UNESCO's 'Man and the Biosphere Programme' in 1971, reflecting its exceptional biodiversity. The area supports the natural presence of 181 seaweed species, while the

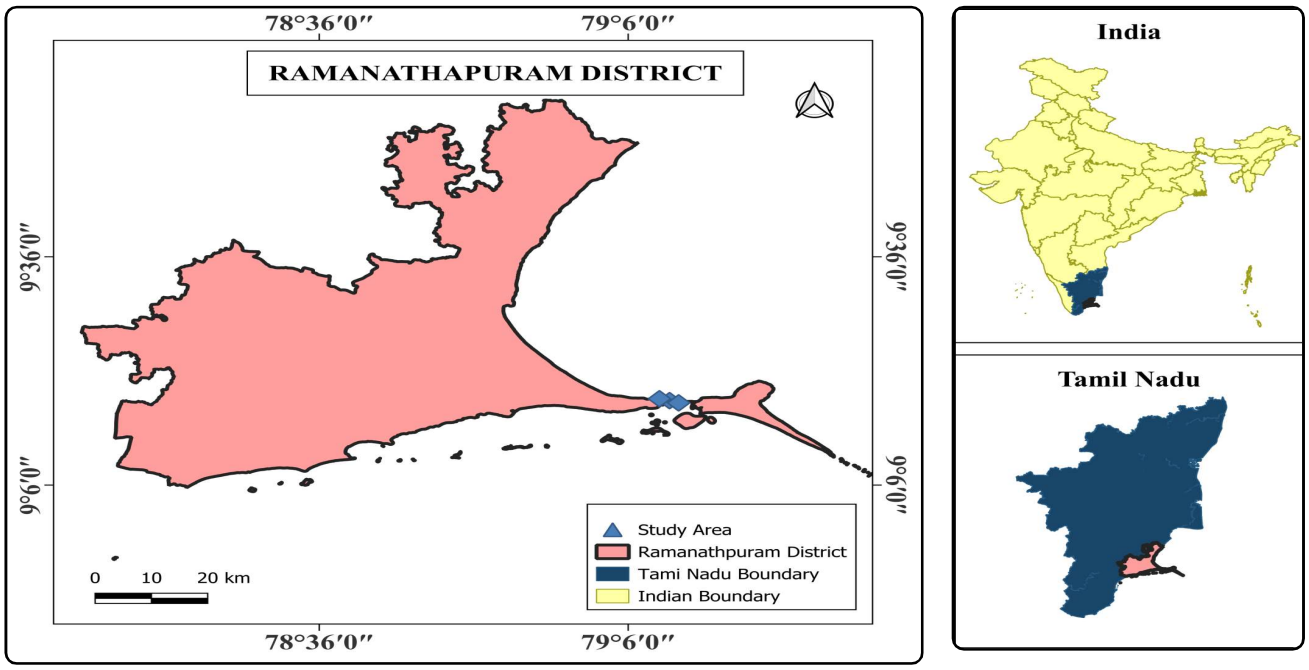

Fig. 1 : Map of study area in Palk Bay, Tamil Nadu

Palk Bay region contributes significantly with 143 species of marine flora, including red, green and brown algae, as well as seagrasses (Ganesan *et al.,*2019).

The Ramanathapuram District was purposively selected as the study site since it lies at the interface of both these ecologically rich marine zones and hosts several seaweed farming clusters. Major farmed species include *Kappaphycus alvarezii*, which are cultivated using floating raft systems.

Seaweed cultivation enables controlled and planned production cycles. Cultivation typically occurs year-round, depending on seedling availability and ocean conditions, with peak harvesting and drying phases occurring during dry months to ensure higher product quality.

Research Methods

The study is based on primary data collected during the calendar year 2024, with fieldwork conducted during May, 2023 and December, 2024. A structured interview schedule and focused group discussions (FGDs) were used to collect detailed insights from key actors across the seaweed value chain, the data collected from 120 Seaweed cultivators, 5 middlemen's, 10 processor's, 20 wholesaler's, 25 retailers and 60 consumers. The tools were pre-tested and refined to ensure clarity, accuracy and contextual relevance.

Analytical Tools

The collected data was analysed with both descriptive and functional methods.

Descriptive statistics : were applied to describe socio-economic characteristics of respondents and marketing practices.

Marketing Efficiency Index (MEI) : was used to evaluate the efficiency of different channels.

$$\text{Marketing Efficiency} = \frac{\text{Consumer Price}}{\text{Marketing Cost + Marketing Margin}} \times 100$$

Price Spread Analysis : was used to determine to examine the distribution of consumer rupee across different intermediaries.

Price spread = Consumer Price - Producer Price

Rank-Based Quotient (RBQ) : Method was used to priorities constraints faced by stakeholders.

$$RBQ = \left(\frac{\sum f_i (n + 1 - i)}{N \times n}\right) \times 100$$

Where :

f_i = frequency of the i^{th} rank

n = total number of ranks

N = total number of respondents

Results and Discussion

The socio-economic profile of the sample respondents reveals diverse demographic characteristics influencing seaweed farming practices. As shown in Table 1, the majority of respondents belong to the middle age group (36-50 years), comprising 38 per cent of the total sample. This is followed by the old age group (above 50 years) at 32 per cent and the young age group (up to 35 years) at 30 per cent, indicating a balanced participation across age ranges with a slight dominance of economically active middle-aged individuals.

Education levels indicate that 42 per cent of respondents are illiterate, while 32 per cent have attained only primary education. Those with secondary education account for 23 per cent, 17 per cent have completed pre-university courses (PUC) and a small fraction (6%) hold a degree. This suggests that seaweed farming is largely practiced by individuals with limited formal education, highlighting the importance of hands-on learning and extension support.

The data of family size shows that 52 per cent of respondents belong to small families (≤ 4 members), 38per centto medium-sized families (5-7 members)

and 10per centto large families (> 7 members), indicating access to a moderate family labor force that impacts farming operations. Regarding experience, 40 per cent of farmers have 15-25 years of experience, forming a strong base of moderately experienced cultivators. Additionally, 34 per cent have less than 15 years of experience, suggesting a significant influx of new entrants, likely driven by recent government initiatives. Smaller groups (16%) have 26-35 years of experience and only 10 per cent have over 36 years of experience in seaweed farming (Kalumanga, 2024).

Table 1

Socio-economic characteristics of sample respondents

Particulars	Number (n = 120)	Per cent
Age group		
Young age (Up to 35 years)	32	30
Middle age (36 - 50 years)	63	38
Old age (Above 50 years)	35	32
Education level		
Illiterate	42	42
Primary	32	32
Secondary	23	23
PUC	17	17
Degree	6	6
Family size		
Small (dH 4 members)	62	52
Medium (5 - 7 members)	46	38
Large (> 7 members)	12	10
Experience in farming (years)		
<15 years	41	34
15 - 25 years	48	40
26 - 35 years	19	16
≥ 36 years	12	10

Seaweed Value Chain

The seaweed value chain in the study involves several groups, each performing different functions from the farm to the consumer (Fig. 2). The chain starts with seaweed cultivators cultivating *Kappaphycus alvarezii*, who are the primary producers. The journey of seaweed from the ocean to the market is a long and layered process. It all starts with the cultivators, who put in the hard work of harvesting, drying and bundling the raw material. Right at this first stage, the pay is low. The real hope for making a decent income hinges on what happens next, actually getting their product to market. This is where middlemen come in, managing the second stage. These middlemen buy up seaweed from many

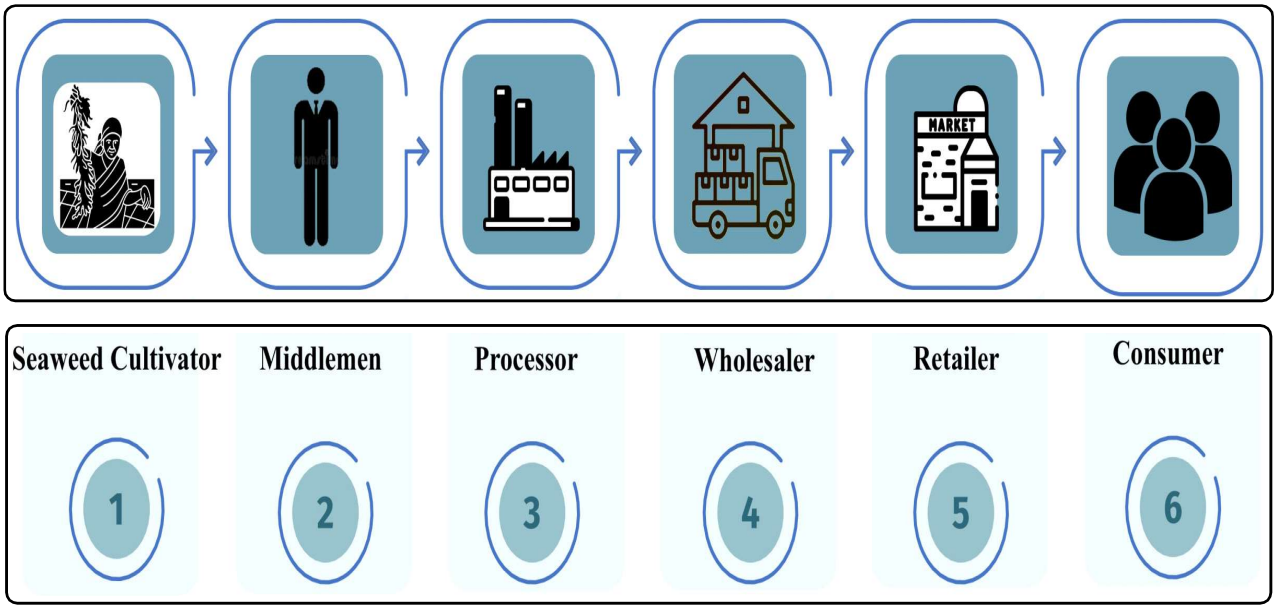

Fig. 2 : Seaweed value chain

different farmers. They take charge of the logistics transporting, sorting and combining smaller batches into larger, more marketable quantities. While their role is crucial because they connect small-scale farmers to bigger buyers, there's a downside. The farmers lose a chunk of their potential earnings to these intermediaries (Naik, 2022).

Processing is the most important stage for value addition in the seaweed supply chain, where the raw seaweed is transformed into something valuable. It's cleaned, graded and turned into products like gel, powder or bio-fertilizer. This isn't a simple task, it requires serious investment in machinery and labor. Because of that, the processors command a much larger slice of the final selling price. This isn't unique to seaweed, we see the exact same trend in other marine and agricultural industries across India the processor usually holds the pricing power. From there, the baton passes to wholesalers. They handle bulk distribution and storage, making sure there's a steady supply ready for local and regional markets. Sure, their involvement adds another layer of cost, but it also creates a reliable pipeline.

Retailers are the final stage that supply products to consumers. They break down those large quantities into the small, accessible packages we see on shelves. Because they're the ones dealing directly with customers, they take a pretty sizable margin for their efforts and it all ends, of course, with consumers. Ultimately, our demand is what drives the entire system, influencing prices and activity at every single step that came before. When you step back and look at the whole picture, a clear and frustrating imbalance emerges. The cultivators are the very foundation of this entire valuechain, but they receive only a tiny fraction of the final consumer's rupee. Meanwhile, processors and retailers, who add the finishing layers of value and own the customer relationship, capture a much larger share. It's a story that plays out across many of India's commodity sectors.

Seaweed Marketing Channels

In the study area, seaweed moves to consumers through five different marketing channels (Fig. 3). These channels vary in terms of the number of intermediaries involved and the extent of value addition. This directly affects both marketing

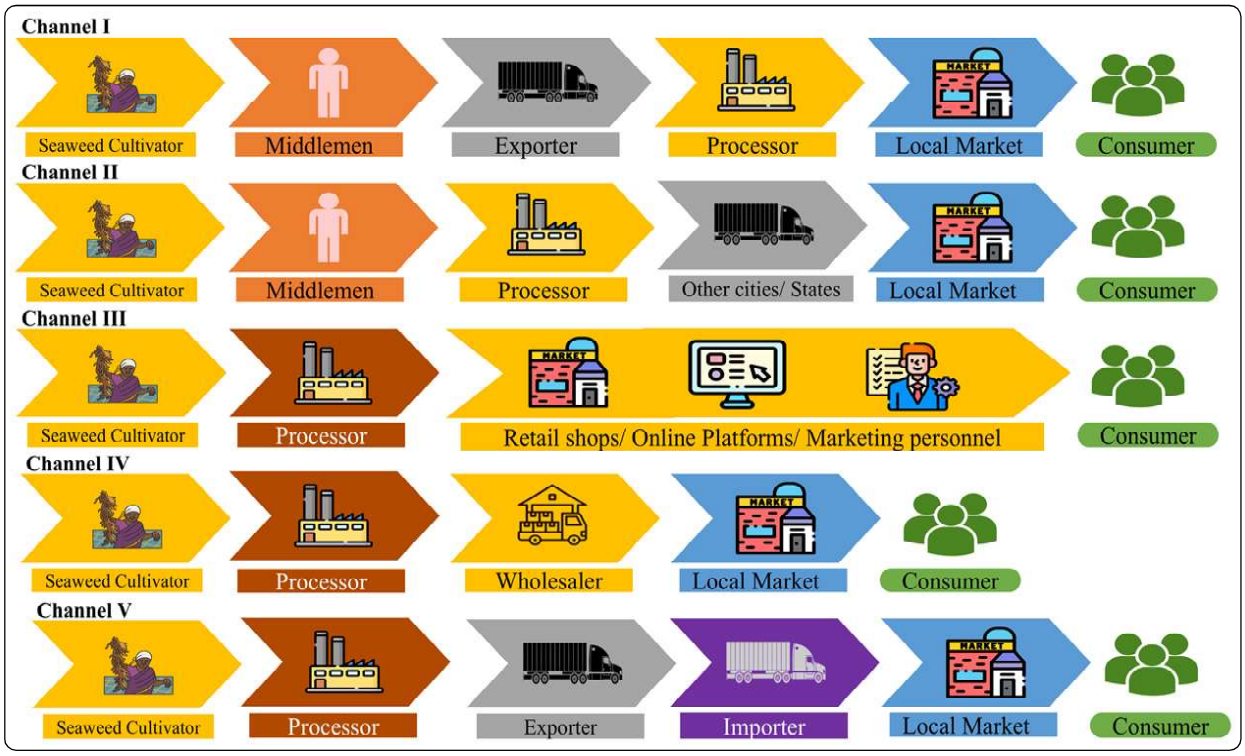

Fig. 3 : Channels in the seaweed Value Chain

efficiency and the share of returns received by the cultivators.

Channel I : This channel turned out to be the longest, most winding road for the seaweed and the least profitable for the growers. The product gets passed from the farmer to an middlemen's, then to an exporter and a processor, before it finally lands in local shops. With so many hands in the pot, costs stack up at every turn. By the end of it all, the farmer is left with just a tiny sliver of what the consumer finally pays.

Channel II : This channel operates in a similar wayas channel I. The main difference is the produce gets shipped to markets in other states. While this gives farmers a wider reach, they're still stuck relying heavily on those middlemen, which keeps their profits frustratingly low.

Channel III : This channel follows a much shorter and more direct route. Here, farmers work directly with processors, who then sell the finished products through their own shops, websites or networks. Cutting out the extra players means farmers can finally negotiate better prices for their work and products get to consumers faster.

Channel IV : This channel is another good option that bypasses middlemen and exporters altogether. In this model, the seaweed goes straight from the farm to a processor and then on to a wholesaler who supplies local markets. Sure, the wholesaler takes their cut, but farmers still end up with a significantly better deal than channel I and channel II.

Channel V : This channel is built for export. Processors sell to exporters, who then work with importers to get the products into foreign markets. While this brings in foreign currency and deals in large volumes, very little of that value trickles down to the farmers themselves. Most of the profit gets captured at the export and import level.

The study clearly shows that shorter supply chains especially channel III and IV are the most efficient. By eliminating unnecessary handling and middle men's, they ensure farmers keep a much larger share of the final price. In contrast, the longer, more complex chains like channel I and V are far less rewarding, as each additional link takes a bite out of the farmers' earnings. This pattern is something we see again and again, not just in seaweed but across India's agricultural and fishing industries, the most direct route is almost always the best one for the producers (Shijagurumayum *et al.,* 2024).

Marketing of Seaweed

The marketing pattern of seaweed among the sampled farmers is presented in Table 2. The results show that seaweed in the study area is marketed through five major channels, with differences in the number of participants and the volume of sales. Among these, channel III and channel IV were the most preferred marketing routes, each followed by 25 per cent of the farmers. These channels were mainly local supply routes, where cultivators sold their harvest directly to nearby processors or wholesalers, ensuring faster payments and better market participation. The average quantity sold through these channels was around 450 kg per month, indicating both steady market demand and the farmers preference for shorter value chains.

About 20 per cent of the farmers sold their produce through channel II and channel V, accounting for monthly sales of 350 kg and 500 kg, respectively. Channel II involved sales through middlemen or traders to local markets, while channel V represented the export-oriented route, where seaweed was aggregated, processed and shipped to oversea buyers. Although channel V recorded the average quantity sold (350 kg/month), only a limited number of farmers could participate due to strict quality standards, capital needs and processing requirements (Liang *et al.,* 2022).The least preferred route was channel I, adopted by only 10 per cent of the farmers, with an average quantity of 250 kg per month. This channel involved multiple intermediaries, resulting in lower farm-gate prices and delayed payments.

The price spread analysis presented in Table 3, clearly shows how the price of seaweed increases as it moves

TABLE 2

Marketing of seaweed under different channels

(n=120)

Marketing Channel	Percentage of Farmers	Quantity Sold (kg/month)
Channel I	10	250
Channel II	20	350
Channel III	25	450
Channel IV	25	450
Channel V	20	350

from the producer to the consumer through different marketing channels. The producer receives average price of Rs.80 per kg across all channels. the consumer price varies significantly depending on the number of intermediaries involved.

Among all the channels, channel V recorded the highest consumer price of Rs.1,943.68 per kg, as this route includes exporters and importers in addition to domestic processors and wholesalers. The high final price in this channel is due to processing, packaging, documentation and export-related expenses, as well as profit margins at each level.

In the channel III, it involves direct sale from producers to processors and then to retailers, recorded the lowest consumer price of Rs.670.82 per kg. This indicates that shorter marketing chains are more cost-effective and ensure faster product movement with fewer mark-ups.

The intermediate channels, such as channel I and channel II, also show notable price increases. In channel I, the product passes through middlemen, processors, exporters and retailers, resulting in a consumer price of Rs.1,457.62 per kg. Channel II involves middlemen and processors only and the consumer price stands at Rs.1,185.08 per kg. The presence of fewer intermediaries compared to channel I slightly reduces the total spread.

The processor's selling price ranged between Rs.510.15 and Rs.632.32 per kg, depending on the channel, which highlights the value addition that takes place at the processing stage. Channels that include exporters and importers show a significant jump in prices due to international trade and transportation costs.

Marketing Cost of Seaweed

The marketing cost of seaweed varied notably among the five channels studied, depending on the number of intermediaries and the distance between producers and end markets. As shown in Table 4, the total marketing cost was highest in channel V (Rs.814.95/kg), which represents the export-oriented route. This was mainly due to additional expenses on documentation, shipping and customs clearance. The lowest cost was observed in channel III

TABLE 3

Price spread of seaweed marketing channels in India

(per kg)

Particulars	Channel I	Channel II	Channel III	Channel IV	Channel V
Producer	80.00	80.00	80.00	80.00	80.00
Middleman	137.40	135.48	-	-	-
Processor	632.32	617.46	510.15	515.85	513.48
Other State	-	965.20	-	-	-
Exporter	1,204.87	-	-	-	1,025.54
Importer	-	-	-	-	1,630.25
Wholesaler	-	-	-	676.02	-
Retailer	1,457.62	1,185.08	670.82	861.56	1,943.68

TABLE 4

Key marketing indicators across seaweed value chain channels

Key Indicator	Channel I	Channel II	Channel III	Channel IV	Channel V
Total marketing cost (Rs. /kg)	638.36	460.24	304.9	392.31	814.95
Total marketing margin (Rs. /kg)	768.16	674.14	318.82	421.24	1079.71
Price spread (Rs. /kg)	1377.62	1105.08	590.82	781.56	1863.68
Producer's share in consumer rupee (%)	5.49	6.75	11.93	9.29	4.12
Marketing efficiency	1.04	1.04	1.08	1.06	1.03

(Rs.304.90/kg), where seaweed was sold directly to nearby processors with minimal handling and transportation.

The total marketing margin followed a similar pattern. It was the highest in channel V (Rs.1,079.71/kg) and the lowest in channel III (Rs.318.82/kg). The longer channels involved several layers of traders and processors, which increased both operational costs and profit margins at each stage.

The price spread, which indicates the gap between the consumer price and the price received by the farmer, was widest in channel V (Rs.1,863.68/kg) and narrowest in channel III (Rs.590.82/kg). This clearly shows that when more intermediaries participate in the chain, the producer receives a smaller share of the consumer's money (Nunes *et al*., 2024).

The producer's share in the consumer rupee ranged from 4.12 per cent in channel V to 11.93 per cent in channel III. This means that farmers get a better return when they sell directly to processors or wholesalers instead of depending on multiple intermediaries.

The marketing efficiencyvaried between 1.03 and 1.08. channels III and IV recorded the highest efficiency values of 1.08 and 1.06, respectively, indicating that these shorter channels are more cost-effective and beneficial for producers. The results clearly suggest that local, simplified marketing channels reduce costs and help farmers retain a larger portion of the final price (Zakaria *et al.,* 2019).

Constraints Faced by Seaweed Stakeholders

The constraints faced by different stakeholders in the seaweed value chain are presented in Table 5. The results indicate that problems are not limited to farmers alone but are spread across all participants, which affects both production and marketing efficiency. For cultivator, the biggest hurdle was low prices, which scored highest with an RBQ of 90.28. This clearly reflects the weak bargaining power that cultivators have in the market. Seasonal employment (81.25) and the absence of protective gear (77.78) were also major concerns, underlining the difficult and vulnerable conditions under which seaweed workers operate. Other issues, such as poor drying facilities and low yields, directly affected both the quality and quantity of their output (Kotyal *et al.,* 2025).

Middlemen, too, cited low prices as their most pressing challenge (86.11), followed by the lack of proper storage facilities (63.89). Seasonal work and unpredictable weather further added to their difficulties, making their operations unstable and dependent on external factors. Processors faced a different set of problems. Their top concern was the poor quality of raw seaweed (87.38), which limited

TABLE 5
Constraints faced by seaweed stakeholders

Stakeholder	Constraint	Rank	RBQ Score
Cultivators	Low prices	1	90.28
	Seasonal employment	2	81.25
	Lack of protection gear	3	77.78
	Inadequate drying facilities	4	63.57
	Low yield	5	63.00
Middlemen	Low prices	1	86.11
	Lack of storage facilities	2	63.89
	Seasonal employment	3	58.33
	Weather conditions affecting supply	4	35.00
Processors	Low-quality raw materials	1	87.38
	Inadequate supply of raw material	2	63.33
	Inconsistent supply from cultivators	3	54.17
	Agar/alginate yield is low	4	54.17
Wholesalers	Price fluctuations	1	79.50
	Inconsistent supply of seaweed products	2	65.30
	Lack of bulk storage facilities	3	58.20
	Dependence on intermediaries for supply	4	51.10
Retailers	Price fluctuations	1	79.20
	Lack of timely market information	2	45.30
	Lack of consumer awareness	3	40.00
	Weather conditions affecting sales	4	30.30
	Social taboos affecting sales	5	60.40

the quality of finished products and reduced t heir market acceptability. They also struggled with inconsistent supply both inadequate (63.33) and irregular (54.17) along with low agar or alginate yield, reflecting the technological and biological constraints they face.

Wholesalers highlighted price fluctuations as their greatest challenge (79.50), along with irregular supply (65.30). Limited bulk storage facilities (58.20) and dependence on inter mediaries (51.10) weakened their ability to maintain a steady flow of products into the market. For retailers, price volatility again emerged as the most serious issue (79.20). Other problems included lack of timely market information (45.30) and low consumer awareness (40.00). Some also pointed to weather conditions (30.30) and even social taboos (60.40) as factors that dampen local demand.

Across the chain, price instability and supply issues were common, though each group faced challenges specific to its role. Farmers bore the brunt of low earnings and poor facilities, processors struggled with raw material quality and supply, while wholesalers and retailers were hit hardest by market fluctuations and limited consumer awareness. Addressing these issues calls for a multi-pronged strategy: improving farm-gate

prices through collective marketing, investing in better drying and storage infrastructure and building stronger consumer awareness around the value of seaweed-based products.

This study shows that seaweed farming in Ramanathapuram district has become a key livelihood activity for small and marginal coastal households. The value chain analysis makes it clear that although cultivators form the base of production, their share in the consumer rupee is still quite low. The largest margins are secured at the processing and retail stages, where most value addition takes place and where direct access to consumers gives higher returns.

Out of the five marketing channels studied, the shorter ones such as channel III and channel IV proved to be more efficient, since they reduce intermediary costs and enable farmers to obtain better prices for their produce. In contrast, export-oriented channels, though linked to higher consumer prices, do not significantly benefit cultivators because much of the margin is absorbed by exporters and importers.

The breakdown of marketing costs and price spread further shows that processors, cultivators and exporters bear the major share of expenses, while middlemen and wholesalers contribute moderately. At the same time, challenges such as low farm-gate prices, seasonal employment, lack of safety gear, inconsistent raw material supply and frequent price fluctuations reduce the overall efficiency of the chain.

For the sector to become more sustainable and equitable, it is necessary to strengthen farmer incomes through collective marketing, improved storage and drying facilities and stronger links with markets. Encouraging small-scale processing units at the village level, combined with digital and cooperative marketing platforms, would allow cultivators to retain a larger share of the value. Tackling these issues would not only improve the earnings of coastal households but also enhance the competitiveness of India's seaweed sector.

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Can Mining and Agriculture Coexist? Assessment of Technical Efficiency and Health outcomes in Singareni Coal Fields, Telangana

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Abstract

This study examines the impact of coal mining on agricultural productivity and human. This study assesses the impact of coal mining on agricultural productivity and human health in a major mining region of Telangana, India. Primary data were collected from 240 farm households, including 120 from four mining-affected villages and 120 from three control villages in the Kakatiya Khani (OC-II) area of the Godavari Valley Coalfields, Bhupalpally district.Bhupalpally district. A structured interview schedule was employed to gather detailed household-level information. The analysis focused on paddy cultivation, cost structures, and net returns, along with an assessment of health outcomes using a Probit regression model. Findings revealed that hired labour accounted for the largest share of cultivation costs in mining-affected areas (₹ 23,344.28; 26.15 %), largely due to land displacement and labour migration. A t-test showed a statistically significant difference in labour usage ($t = 2.3$; $p < 0.05$), and paddy yields were significantly lower in mining regions. Technical efficiency was estimated using a bias-corrected Bootstrap Data Envelopment Analysis (DEA), yielding a mean efficiency score of 0.70 indicating that inputs could be reduced by 30 per cent without compromising on output. While most farmers (72 per cent) had efficiency scores between 0.70 and 0.80, 32 farmers achieved full efficiency under the radial model. Health analysis revealed that education significantly reduced the likelihood of respiratory illness, while employment in mining activities increased it. Improved water quality and higher income were associated with better health outcomes. The model's predictive accuracy was strong (AUC = 0.9588). These findings emphasize the need for integrated policy measures to protect environmental quality, strengthen health systems, and promote resilient farming practices in mining-affected regions.

Keywords : Coal mine, Paddy, Respiratory illness, Technical efficiency, Cultivation cost

The growing population has intensified the global demand for goods and services, prompting governments to expand industrial sectors. In this context, mineral resources particularly coal play a vital role in driving economic development, especially in countries like India. Coal remains the dominant fossil fuel worldwide and is projected to continue leading the global energy mix for decades (Anonymous 2015). In India, coal accounts for about 55 per cent of total energy production and fuels over 70 per cent of electricity generation. As one of the world's largest coal producers, India depends heavily on this resource to meet its rising industrial and domestic energy needs. Coal production has surged from 382.62 million tonnes (MT) in 2004-05 to 893.19 MT in 2023-24, with projections reaching 1,100 MT by 2024-25 (Anonymous 2024a). Over the past decade, production has grown by 57.87 per cent. The coal mining sector contributes significantly to India's economy through royalties,

taxes and export earnings amounting to approximately USD 184.4 million. Mining and quarrying contribute about 2.5 per cent to India's GDP. Under the Index of Industrial Production (IIP), the mining sector recorded 1.7 per cent annual growth. As India aims for a $5 trillion economy by 2024-25, mining is set to play a crucial role in achieving robust economic growth. The extraction of mineral resources contributes not only to economic growth but also to the development of local communities. It facilitates the establishment of schools, hospitals, roads and communication networks. Revenues earned by state governments from mining are often reinvested in welfare programs to enhance socio-economic well-being (Li *et al*., 2011). However, despite its economic benefits, mining also causes serious environmental damage, including deforestation, resource depletion and pollution of water and soil. These impacts threaten local ecosystems, reduce agricultural productivity and harm community health. Thus, mining poses significant challenges that must be managed for sustainable development (Mishra and Punjari, 2008).

Air pollution affects agriculture in multiple ways, often reducing crop yields and nutritional quality. It damages crops by lowering productivity or degrading produce quality resulting in output decline even when input levels remain constant. The most harmful air pollutants include sulfur and nitrogen compounds, photochemical oxidants, ozone and suspended particulate matter (SPM), all of which contribute to economic losses in agriculture, an increasing concern globally. In mining areas, elevated levels of SPM pose serious challenges, as coal dust settling on crops interferes with nutrient absorption, photosynthesis, and productivity (Li *et al.,* 2011). The study showed that villages near coal mines experienced reduced rice yields due to high coal dust concentrations. Sulfur in the dust disrupts crop respiration and forms acidic compounds upon contact with moisture, damaging leaves and reducing yield. In such regions, SO, emissions alone caused an estimated 2.50 per cent loss in agricultural output.

Moreover, mining-induced pollution severely affects human health. Residents in these areas commonly suffer from respiratory illnesses, skin problems and waterborne diseases, often with limited access to healthcare (Hota and Behera, 2015). Open-pit mining releases pollutants such as SPM, RSPM (Suspended Particulate Matter and Respirable Suspended Particulate Matter), ozone, sulfur oxides and nitrogen oxides, increasing health risks (Atkinson and Hamilton, 2003). Mining is among the most hazardous occupations, causing both short and long-term health effects, including cancer and respiratory diseases. People living near coal mines face higher risks of heart disease, hypertension and cancer (Hendryx *et al.,* 2008 and Hendryx & Ahern, 2008) and often consume chemically contaminated water. Occupational diseases like silicosis and coal workers' pneumoconiosis (CWP) are common among miners (Schatlez & Stewart, 2012; Parette *et al.,* 2017 and Lechner *et al.,* 2016). Heat stress and high humidity further reduce worker productivity. Despite growing awareness, limited research explores how mining-induced pollution impacts both agriculture and community health. This study aims to fill that gap, focusing on coal mining regions in Telangana, India.

The Singareni Collieries Company Limited (SCCL) has been engaged in coal exploitation for over 125 years. Of the 470 km-long Pranahitha-Godavari Valley Coalfields (PGVCF), a substantial 350 km stretch is located primarily within the southern Indian state of Telangana. To meet the increasing demand for coal, exploration activities have been intensified in deeper areas as well as in previously unexplored coal-bearing zones of the PGVCF. Regional exploration was initially conducted by the Geological Survey of India (GSI), with detailed exploration subsequently carried out by SCCL between 1980 and 1983. (Anonymous, 2024 b). The proposed KTK OC-2 (Khakatiya Khani Open Cast - 2) Project (comprising KTK-2 and 2A Inclines) is located adjacent to the existing KTK OC-1 Project under the Bhupalpally Block-I. The KTK OC-1 Project had already exhausted its coal reserves, with its operational lifespan coming to an end. To meet the increasing demand for coal, the KTK OC-2 Project was initiated in 2014-15. Exploration efforts identified 1,292 million tonnes of coal reserves within an area of 82 square kilometres in the Mulug

Coal Belt, located at shallow depths, making them suitable for opencast mining operations.

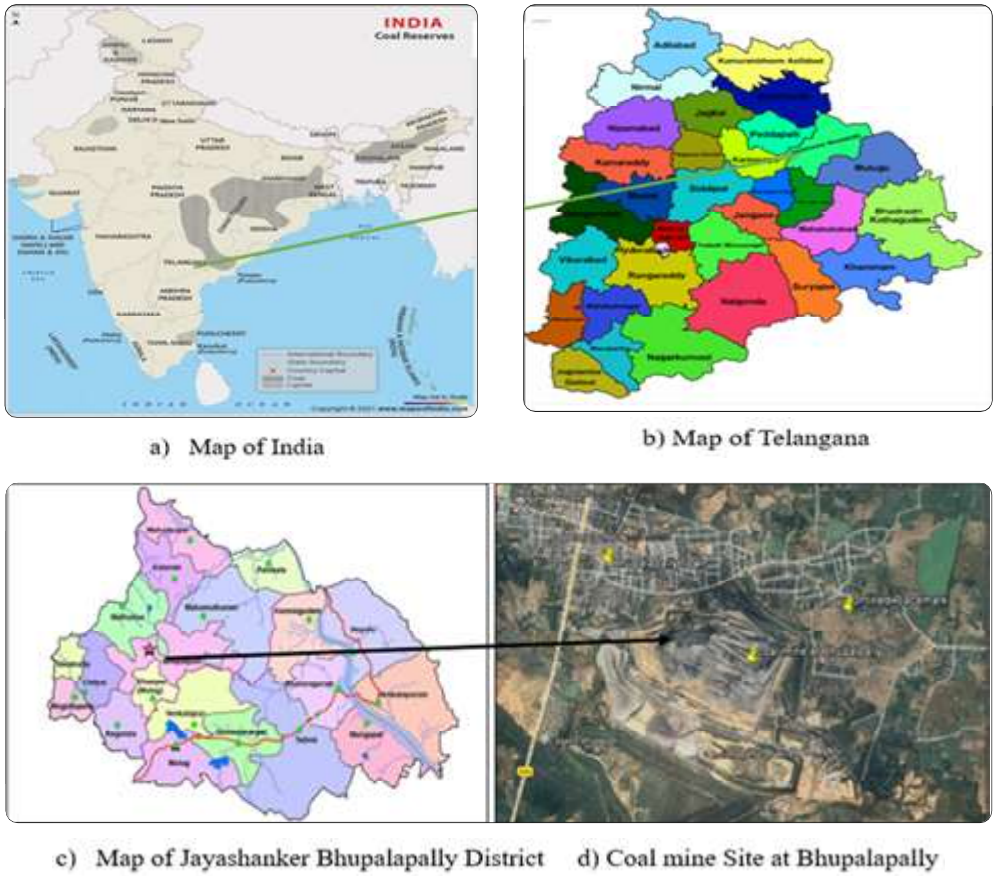
Methodology

Study Area

The project is situated in the Jayashanker Bhupalpally district, formerly known as Kakatiya Khani - Open Cast II. It is positioned between the coordinates N 18°26'41.6704" to N 18°28'7.0715" latitude and E 79°50'16.1445" to E 79°52'36.3261" longitude, approximately 260 km away from the state capital.

The Bhupalapally district, situated in the northern part of Telangana, has been heavily reliant on coal mining as a primary economic activity for several decades. This region hosts two prominent opencast coal mines: OC-1, which was operational for many years, and OC-2, which began excavation in 2015. OC-1 was the first large-scale opencast mine in the area and reached its peak production at various points during its operational years. However, as mining reserves dwindled, the lifespan of OC-1 came to a close, and by 2015, it ceased operations. The transition to OC-2 marked a new era in coal mining, with the latter featuring larger scale operations and advanced machinery that allowed for increased coal extraction.

The study adopted a purposive sampling technique for the selection of respondents. A total of 120 sample respondents were selected from mining-affected and control villages. Four villages located within the mining influence zone (within a radius of 5 km to 13 km from the mining area) were selected to represent mining-affected regions. For comparison, three villages situated outside the immediate mining influence were selected as control villages. The selection was based on proximity to mining



a) Map of India b) Map of Telangana

c) Map of Jayashanker Bhupalapally District d) Coal mine Site at Bhupalapally

Fig. 1 : Coal mine site at Bhupalapally

Table 1

Particulars of selected mandals, villages, farmers in the Singareni mining region of Bhupalpally District

District	Mandal	Mining Villages	No. of samples
Bhupalpalli	Bhupalpalli	Gaddiganapally	30
		Jangedu	30
		Khasimpally	30
		Velishalapally	30
		Sub total	120
Bhupalpalli	Bhupalpalli	Gollapally	40
	Koyyuru	Tadwai	40
		Thumdla	40
		Sub Total	120
	Total Sample Respondents		240

operations, degree of exposure to mining externalities, and willingness of households to participate in the survey, ensuring reliability and comparability of data. The study intentionally focused on a single district, particularly villages located within 5 km and 13 km radius of the mining area, to capture the localized and intensity-specific impacts of coal mining on agriculture and livelihoods. Mining impacts such as air pollution, land degradation, groundwater changes, and health externalities are highly spatial in nature and diminish significantly with increasing distance from the mine. Therefore, selecting mandals beyond the defined proximity would introduce substantial agro-ecological and socio-economic heterogeneity, making it difficult to isolate mining-induced effects The sampling design followed a comparative proximity-based framework, wherein four mining-affected villages were selected within the impact zone and three control villages were chosen outside the immediate mining influence. The selection of control villages was constrained by field realities. Several villages were either unwilling to participate due to apprehensions associated with ongoing mining activities or were themselves influenced by newly initiated open-cast mining operations, which could bias counterfactual comparisons. Hence, only villages meeting the criteria of minimal mining exposure and willingness to provide reliable information were included.

Thus, the chosen sampling structure ensured data reliability, comparability, and methodological consistency while minimizing contamination of the counterfactual group.

Analytical Tools

Sampling Method : A purposive random sampling technique was employed to select the study area and respondents. Jayashankar Bhupalpally district was purposively selected due to its long-established coal mining operations. Within the district, Bhupalpally mandal and Koyyuru mandal were selected because of their proximity to the mining area operated by Singareni Collieries Company Limited. From these mandals, four mining-affected villages and three control villages were identified. A total sample of 240 farm households was selected, comprising 120 households from mining villages and 120 households from control villages. Within each village, respondents were selected using simple random sampling to ensure representation of households with diverse socio-economic and farming characteristics. Primary data were collected for the agricultural year 2023-2024.

Costs and Returns concepts

Cost Concepts : Costs and Return analysis, Descriptive statistics, including mean values, percentages, and *t-tests*, have been used to assess the significant differences in the costs and returns of agricultural production between the mining and controlled regions (Sen and Bhatiya, 2004).

Return concepts

Gross returns : Per hectare gross returns were calculated by using the below formula

Gross Returns = Yield per hectare x Selling Price

Net returns : It is the Gross Returns minus total cost of cultivation which include Total Variable Costs and Total Fixed Costs

Net returns over cost of cultivation = GR - TC (TVC + TFC)

Where GR is Gross Returns, TC is the total costs, TVC is Total Variable Cost and TFC is Total fixed costs.

$$\textit{B-C Ratio : } BCR = \frac{\text{Sum of present value of benefits}}{\text{Sum of present value of costs}}$$

Technical Efficiency

Technical Efficiency (TE) assesses how well a Decision-Making Unit (DMU) converts inputs into outputs relative to the best performer. TE can be measured using parametric, non-parametric and machine learning methods. Parametric models like the Translog Production Frontier use regression and assume a specific functional form, suitable for panel data. Non-parametric approaches like Data Envelopment Analysis (DEA) require fewer assumptions and use linear programming to compare each unit against the most efficient peer. This study uses Bootstrap DEA with an input-oriented Variable Returns to Scale (VRS) model, considering inputs like fertilizers, labor and saplings, with yield as the output, to estimate bias-corrected efficiency scores.

$$TE_i = \min_{\theta i\ \lambda} \theta_i \quad \text{.....1}$$

Subject to,

$$-Y_i + Y\lambda \leq 0,$$

$$\theta_i X_i - X\lambda \geq 0$$

$$\lambda \geq 0$$

$$\sum_i^N \lambda_i = 1$$

Technical Efficiency (TE) measures how effectively a Decision-Making Unit (DMU) transforms inputs into outputs relative to the best performer. This study uses an input-oriented Variable Returns to Scale (VRS) Data Envelopment Analysis (DEA) model to assess TE, considering inputs like FYM, fertilizers, plant protection chemicals, bullock and machine labor, human labor, and saplings, with yield as the single output. The Bootstrap DEA method is employed to obtain bias-corrected efficiency scores by resampling the data and simulating the production process. This non-parametric approach allows efficiency estimation without assuming a specific functional form, making it suitable for analyzing diverse agricultural practices.

The non-radial measure of technical efficiency (also called slack-based measure) corresponds to the mathematical properties expressed as:

$$min_v = \frac{1 - (1/r)\sum_{i=1}^{r} S_i^- / X_{ik}}{1 + (1/t)\sum_{n=1}^{t} S_n^+ / Y_{nk}} \quad \text{.....2}$$

Subject to,

$$X_{ik} = \sum_{j=1}^{n} x_{ij}\delta_j + S_i^-, \quad i = 1, \ldots, r$$

$$Y_{nk} = \sum_{j=1}^{n} Y_{nk}\delta_j - S_n^+, \; n = 1, \ldots, t$$

$$\delta_j \geq 0, \; j = 1, \ldots \ldots, n$$

$$S_i^- \geq 0, i = 1, \ldots \ldots r$$

$$S_n^+ \geq 0, n = 1, \ldots \ldots t$$

Where, *v* denotes non-radial technical efficiency that relates to agriculture farms as DMUs connected with the t set of output Y_{nk}(s= Agriculture Production in terms of yield *i.e.*, Paddy, Cotton and Chilli) and r input set X_{ik} (r = quantity of seed, fertilizers and labour); δ_j is non-negative vector allowing the production possibility set construction for DMUs *j*; n denoting the number of DMUs (j = 1,…n): S_i^- and S_n^+ are slacks in input (x) and output (y) vector with access and shortfall respectively. No input access referred by $S_i^- = 0$ and no output shortfall by $S_n^+ = 0$.

Prior studies were found to employ Tobit (following Tobin 1958) or Ordinary Least Square (OLS) regression to estimate the causal effect of TE scores on explanatory variables. First, TE scores are estimated through the conventional DEA model, and second, they are regressed on the selected explanatory variables as per following process:

$$\theta_i = \alpha + \beta_i z_i + \alpha_i \quad \text{.....3}$$

Where, θ_i represents the technical efficiency score, z_i are the explanatory variables influencing technical efficiency scores of the agriculture farms, β_i are the parameters to be estimated and α_i the standard error term distributed normally and independently. However, conventional two step DEA procedure involves problems related to lack of well-defined data-generating process along with misleading inference. To examine the socio-economic and environmental factors influencing farm-level technical efficiency, the Simar and Wilson (2007) double-bootstrap procedure was applied. This method corrects bias in TE scores and enables statistically valid inference in the second-stage regression.

The second-stage truncated regression includes explanatory (inefficiency) variables such as:

- Age of the farmer
- Years of Education
- Farming Experience
- Mining Status (dummy variable: 1 = mining region, 0 = non-mining region)

These variables are hypothesized to influence the inefficiency term. The analysis was performed on the combined sample of mining and non-mining farms, allowing the effect of mining on efficiency to be isolated while controlling for other household-level characteristics. The present study followed Badunenko and Tauchmann (2019) to estimate the bootstrap DEA by integrating the estimation of TE scores and its determinants. Here, first four steps show the estimation of bias corrected efficiency score and remaining three steps show the estimation of truncated regression that identify the variables influencing the technical efficiency score of agriculture farm.

Step 1 : Estimate the TE score (θ_i for all agriculture farms *i* = 1……n following the Equation (1)

Step 2 : Obtain the coefficient estimates of $\hat{\beta}$ and variance parameter ($\hat{\delta}$) in truncated regression of θ_i on z_i using maximum likelihood method.

Step 3 : Repeat bellow steps (Step 'a' to 'd') 2000 times for each *i* = 1…n for generating bootstrap estimates $\hat{\theta}_i^b$ where b = 1.....B_n

a) An artificial error term $\hat{\alpha}_i$ is drawn from the truncated distribution for each *i* = 1……n

b) Artificial efficiency scores $\hat{\hat{\theta}}_i = z_i\hat{\beta} + \hat{\hat{\alpha}}_i$ are computed for each *i* = 1……n

c) An artificial data set is constructed with input quantities $\left(\hat{x}_i = {}^{\theta_i}/_{\hat{\theta}_i}\right) x_i$ and output quantities ($\hat{y}_i = y_i$)

d) The artificial data set generated in (c) and Equation (1) provides a reference data set in DEA that generates the artificial efficiency score estimates $\hat{\theta}_i^b$ for each original decision-making units (DMUs) *i* = 1……n)

Step 4 : Calculate the bias corrected efficiency scores $\hat{\theta}_i^b$ $\hat{\theta}_i - (\frac{1}{B_i}\sum_{b=1}^{Bn} \hat{\theta}_i^b - \hat{\theta}_i$ as) for each *i* = 1…n

Step 5 : Using maximum likelihood method run a truncated regression of $\hat{\theta}_i^b$ on z_i to obtain coefficient estimates of $\hat{\hat{\beta}}$ and a variance parameter estimates $\hat{\hat{\delta}}$.

Step 6 : Repeating the below steps (Step 'e' to 'g') with B_m = 2000 times to obtain bootstrap estimates $(\hat{\beta}^b, \hat{\alpha}^b)$ with b = 1….. B_m

a) An artificial error $\hat{\alpha}_i$ is drawn out of the truncated distribution for each *i* = 1……n

b) Artificial efficiency scores $\hat{\theta}_i = z_i\hat{\beta} + \hat{\alpha}_i$ are computed for each *i* = 1……n

c) Using maximum likelihood method, a truncated regression is run for $\hat{\theta}_i$ on z_i to get the bootstrap estimates $\hat{\beta}^b$ and $\hat{\delta}^b$.

Step 7 : Construct confidence intervals and standard errors for β and δ using bootstrap estimates and and estimates of and (obtained in Step 5). The (1- per cent confidence interval of i^{th} elements of vector is constructed as - in a way that the computed confidence interval for is.

The estimation of the double-bootstrap DEA is carried out in Stata17 software (Statacorp, college station, TX, USA) following the 'simarwilson' command with base as input-oriented VRS approach.

Probit Model

The Probit model is used to identify and analyse the factors that influence the occurrence of respiratory illness in the study population. This binary dependent variable is modelled as a function of several independent variables, including gender, age, education, mine job exposure, water quality, household size, and income (Hota and Behera, 2015). The model can be expressed as:

In this analysis, the dependent variable (Yi) is Respiratory illness occurrence of the sample farmers. If Yi is the random variable (dichotomous), it can be assumed that Y takes the vales 1 or 0, where 1 represents presence of respiratory illness and 0 otherwise. If Xi……Xn are explanatory variables to be the factors determining the respiratory illness, then the probit model specifies that the conditional probability of event (*i.e.*, that Y = 1) given the values of Xi……Xn is as follows

$$P(Yi) = \frac{1}{1 + exp - (\alpha - \sum \beta i Xi} \quad \ldots . (1)$$

In order to linearize the right-hand side, a log transformation was applied by taking the logarithm on both sides:

$$Probit\ P(Yi) = \alpha + \sum \beta i Xi + \varepsilon \quad \ldots . (2)$$

Where, Yi = 1, if the farmers reported respiratory illness, are and 0 otherwise α = Constant / intercept term Xi = Independent variables for i = 1 to n βi = coefficients for the ith independent variables

Output elasticities

Then marginal effects of the explanatory variables at the mean could be obtained by:

$$\text{Marginal effect of } X_i = \frac{dy}{dXi} * \frac{\overline{Xi}}{\overline{Y}} \text{ or } b_i * \frac{\overline{Xi}}{\overline{Y}} \quad \ldots .3$$

Where,

bi = Parameter estimate / partial elasticity associated with each independent variable)

X = Mean of independent variable

Y = Mean of dependent variable

The variables for the factors determining respiratory illness and their expected sign *i.e.,* directional relationship are given below

Table 2

Description of the variables for the factors determining Respiratory Illness

Variables	Description	Expected Sign
Gender (X_1)	(Male 1, Female 0)	+
Age (X_2)	Age 1 for the individuals when age is above 50 and below 15 and other 0)	+
Education (X_3)	No of the years of schooling	-
Mine Job (X_4)	(Occupation working in the mine 1, If not 0)	+
Water quality (X_5)	(Respondents reporting good water quality 1 if not 0)	-
Household size (X_6)	No. of people living in a house	+
(Income) (X_7)	Family Income	-

Results and Discussion

The study reveals that labour and material costs are the primary contributors to the overall cost of paddy cultivation in both mining and controlled regions. However, significant differences exist between these regions due to mining-related disruptions. Labor costs are one of the highest contributors to cultivation expenses. In the mining region, total labour costs amount to Rs.51,270.61, which is significantly higher than Rs.41,454.53 in the controlled region t-value is 1.62. Hired labour costs are particularly high in mining regions at Rs.23,344.28 compared to Rs.15,864 in the controlled region found that there is statistical difference between mining and controlled region (t=1.39) at five per cent level of significance. This is likely due to labour shortages and migration caused by mining activities. Family labour costs are also higher in mining areas (Rs.13,183.53) compared to control areas (Rs.10,772.66) showing that there was a statistically significant difference between mining and controlled region by one per cent (*t* value is 2.90).

The cost on fertilizer is significantly higher in the mining region (Rs.8,523.33) than in the control region (Rs.6,528.33) with t value of 1.49 significant difference by one per cent, reflecting the excessive use of fertilizers due to declining soil fertility in mining areas. Cost on farmyard manure (FYM) are lower in the mining region (Rs.5,210.31) compared to Rs.6,500 in the control region with highly significant difference (-7.51) at 1 per cent, suggesting reduced availability or utilization in mining-affected areas. Seed costs are also higher in the mining region (Rs.2,665.83) than in the control region (Rs.2,123.56) (t = 1.57) with five per cent level, indicating increased input requirements for crop establishment. The total cost of cultivation in mining regions is Rs.89,264.87, which is higher than Rs.82,203.23 in the controlled region (t = 1.45) with five per cent significant level. This increase is primarily due to higher labour and fertilizer costs in mining areas. Gross returns are significantly lower in mining regions (Rs.1,04,971.7) compared to Rs.1,12,754.8 in control regions (t value is 5.23) with one per cent significant level, indicating the adverse effects of mining on productivity. Net returns in the mining region (Rs.15,706.81) are considerably lower than in the control region (Rs.30,551.56) (t = 1.27***), emphasizing the economic burden on farmers in mining-affected areas.

The Irrigation cost was also found to be significantly different between mining and control regions, because coal mining has led to severe groundwater depletion, causing the failure of borewells in mining regions. As a result, farmers rely more on rainfed agriculture or surface water sources, reducing their irrigation costs compared to non-mining areas where borewell irrigation is still functional. However, this lower cost comes at the expense of unreliable water availability, leading to reduced crop yields and increased vulnerability to droughts. The long-term impact of mining on water resources threatens

Table 3

Comparative Economics of paddy production for mining and controlled region in buffer zone of Singareni Coal mining area

Particulars	Mining Region			Controlled Region			
	Quantity (Per ha)	Cost per ha	(%) to CoC	Quantity (Per ha)	Cost per ha	(%) to CoC	t-test
Labour Used							
Family Labour (days)	23.97	13183.5	14.76	26.93	10772.66	13.1	2.9 ***
Hired Labour (days)	42.44	23344.28	26.15	39.66	15864	19.3	2.3 **
Bullock Labour (pair)	1.5	250.33	0.28	1.18	255.62	0.31	0.67 Ns
Machinery cost (hr)	9.35	14492.5	16.23	10.35	14562.25	17.71	0.47 Ns
Total Labour Costs		51270.61	57.43		41454.53	50.43	1.62 **
Material Costs							
Seeds (Kg)	66.41	2665.82	2.98	62.35	2123.56	2.58	1.57 **
FYM (t)	2.6	5210.313	5.83	3.59	6500	7.91	-7.5 ***
Fertilizer (Kg)	452.33	8523.33	9.54	335.28	6528.33	7.94	1.49 **
Plant Protection (L)	0.39	4558	5.1	0.91	4799.32	5.84	0.38 Ns
Weedicides (L)	0.25	2653.25	2.97	0.19	2258.95	2.75	0.75 Ns
Irrigation Costs per ha		3766.25	4.21		4985.65	6.07	1.93 **
Total Material Costs		27376.97	30.66		27195.81	33.08	0.95 *
Total Working capital		78647.58	88.1		68650.34	83.51	
Interest on working capital (7%)		1916.38	2.14		4805.524	5.85	
Total Variable costs		80563.97	90.25		73455.86	89.36	3.25 ***
Fixed cost							
Land Revenue	30	0.03			30.26	0.04	0.01 Ns
Depreciation	1156.33	1.29			1256.32	1.53	0.62 Ns
Rental value of land	6582.33	7.37			6523.57	7.94	1.23 Ns
Interest on fixed capital (12%)	932.23	1.04			937.218	1.14	
Total fixed costs	8700.89	9.74			8747.368	10.64	0.12 Ns
Total costs of cultivation	89264.87	100			82203.23	100	1.45 **
Cost of production (Per Quintal)	2253.57				2257.78		
Marketing costs (Rs/ha)	2045.17				2280.83		
Bags	49.6	742.5			55.23	552.3	
Packaging and transportation		452.32			276.15		
Commission per marketing charges	850.35				1452.38		

Note : (*, **, *** Significance at 10 per cent, 5 Percent and 1 Per cent and Ns is Non-Significant)

agricultural sustainability, making farmers increasingly dependent on external water sources or government interventions for irrigation support. because findings were similar in lines with Gururaj and Mahadeviah, (2018). In controlled areas, better water availability leads to higher irrigation investment and costs. However, total variable costs (t-test = 3.25) are significantly higher in mining regions due to environmental degradation, which increases input needs. Labour, a major expense in paddy farming, adds to these costs, especially when used inefficiently, as noted by Sri *et al.* (2022) and Gururaj and Mahadeviah (2018). To reduce costs and improve sustainability, efficient labour practices and crop diversification such as millet cultivation in low-irrigation zones-are essential (Satishkumar and Umesh, 2017; Pavithra and Gaddi, 2022 Amrutha, and Chandrakanth, 2018)).

The comparison between mining and controlled regions shows clear differences in agricultural performance (Table 4). Main product yields were lower in the mining area (46.58 q/acre) than in the controlled region (49.95 q/acre), with a significant t-test value of (3.25**). Gross and net returns were also higher in the controlled area (Rs.1,12,755 and Rs.30,551/acre) compared to the mining region (Rs.1,04,972 and Rs.15,706/acre), with gross returns showing a highly significant difference (t = 5.23***). The return per rupee of investment was also better in the controlled region (1.37 vs. 1.14), highlighting the adverse effects of mining on profitability.

Frequency Distribution of Technical Efficiency Scores for Sample Paddy Farmers across Mining and Controlled Regions

The frequency distribution of technical efficiency (TE) scores among 240 paddy farmers, estimated using bias-corrected, radial, and non-radial DEA approaches, reveals varying levels of efficiency. The bias-corrected scores had a mean of 0.70, indicating that inputs could be reduced by 30 per cent without affecting output.

Table 5

Frequency Distribution of Technical Efficiency Scores for Sample Paddy Farmers across Mining and Controlled Regions

TE score	Bias Corrected	Radial	Non radial
0.00 to 0.10	0	0	4
0.10 to 0.20	0	6	6
0.20 to 0.30	0	2	46
0.30 to 0.40	8	6	36
0.40 to 0.50	22	32	64
0.50 to 0.60	24	24	42
0.60 to 0.70	44	18	12
0.70 to 0.80	72	68	10
0.80 to 0.90	64	52	2
0.90 to 1	6	32	18
Total	240	240	240
Mean	0.70	0.72	0.46
Minimum	0.31	0.12	0.070
Maximum	0.92	1	1

Table 4

Returns from the mining and controlled region in buffer zone of Singareni Coal mining area

Particulars	Mining			Controlled region			
	Quantity (Q)	Rate	Value (Rs./ha)	Quantity (Q)	Rate	Value (Rs./ha)	t-test
Main product	46.58	2200	102476	49.95	2200	109901	3.25 **
By product	1.85		2495.68	1.93		2853.74	
Gross returns			104972			112755	5.23 ***
Net returns			15706.8			30551.6	1.27 ***
Returns per rupee of investment			1.148			1.37166	

Note: (*, **, *** Significance at 10 per cent, 5 Per cent and 1 Per cent and Ns is Non-Significant)

The distribution of technical efficiency scores reveals distinct patterns across the three approaches. Most of the Bias Corrected scores are concentrated in the 0.70-0.90 range about 57 per centage, indicating a relatively higher level of efficiency among the sampled farmers. Similarly, the Radial scores also cluster in the 0.70-0.90 range around 50 per centage, reinforcing the evidence of moderate to high efficiency in this method. In contrast, the Non-radial scores are skewed towards the lower end, with the largest proportion of farmers i.e., 27 per centage falling in the 0.40–0.50 interval, highlighting greater inefficiencies when assessed through this approach.

Truncated Regression Estimates of Factors Influencing Technical Efficiency in Paddy Cultivation across Mining and Control Areas

Table 6 presents the results of the truncated regression model estimating factors affecting the bootstrapped technical efficiency (TE) of paddy farmers in mining and control regions. The model, supported by a highly significant Wald chi-square statistic (240.56), confirms the joint significance of the explanatory variables. The analysis reveals that the use of chemical fertilizers negatively and significantly impacts TE (significant at 5% level), likely due to inefficient or excessive application, particularly in ecologically stressed mining zones.

Table 6

Truncated Regression Estimates of Factors Influencing Technical Efficiency in Paddy Cultivation across Mining and Control regions

Variable	Coefficient	Bootstrap Standard Error
Seeds	-0.00024	0.00006
Fertilizers	-0.01164 **	0.00044
PPC	-0.17640	0.37797
Organic Fertilizers	0.00993 ***	0.00487
Machine hours	0.00428 **	0.00147
Total labour	0.00228	0.00047
Bullock labour	0.45517	0.10114
Age	0.00857	0.00639
Education	0.00086	0.01869
Farming experiences	0.00125 *	0.00110
Mining status	-0.14991 ***	0.21251
No. of the Decision making Units		240
No. of Bootstrap making Units		2000
Wald chi square		240.56

Table 7

Major Health issues in Mining and controlled region

Particulars	Mining Region			Controlled Region		
	People affected (%)	Average Cost (INR)	Premature Deaths	People affected (%)	Cost per person (INR)	Premature Deaths
Respiratory illness (Asthma, TB and Lung Cancer)	32.5	5747.13	1	20.43	3363.364	-
Myocardial infarction/ Heart issues	3	4582.35	2	2.5	4250.32	1
Skin diseases	12.5	2500	-	7.5	1125	-
Gastric issues	26.67	2100	-	32.5	516.129	
Conjunctivitis	8.33	952.35	-	5	1060.32	
Joint pains	2.5	1589.35	-	4.17	445.18	
Others (viral fevers, flu, Typhoid and Diarrhoea)	13.33	88.88	-	12.5	850.29	

In contrast, the use of organic fertilizers significantly enhances TE (significant at 1 significant level), highlighting their role in improving soil health and reducing chemical dependency. Mechanization also shows a positive and statistically significant effect at the 5 per cent level, indicating improved operational efficiency. Farming experience contributes positively and is significant (10% level). Notably, mining status negatively influences TE (significant at 1% level), emphasizing the adverse impact of mining on agricultural efficiency.

Major Health Issues in Mining and controlled region

Health expenditures and illness rates are higher in mining regions. Respiratory issues affect 32.5 per cent in mining areas with a higher cost (Rs.5747) than 20.43 per cent in controlled regions (Rs.3363). Gastric problems are more prevalent in controlled areas (32.5%) but costlier in mining zones. Premature deaths are slightly higher in heart issues.

Determinants of Respiratory Illness in Mining and controlled regions

The probit model results in Table 8 highlight key determinants of respiratory illness across mining and controlled villages, with a high predictive accuracy (ROC = 0.95). Education shows a significant negative effect (β = -0.634; p = 0.003), indicating that more educated individuals are less likely to suffer from respiratory issues, likely due to better awareness and healthcare access. Employment in mining is strongly associated with higher risk (β = 2.147; p = 0.019), reflecting exposure to pollutants. Better water quality significantly reduces illness probability (β = -1.700; p = 0.009), suggesting indirect health impacts from contamination. Income also shows a protective effect (β = -1.159; p = 0.028), as wealthier households tend to have better living and health conditions. However, gender, age and household size are statistically not significant. Overall, the results emphasize the role of socio-economic and environmental factors in influencing respiratory health in mining regions.

Education may empower individuals with the knowledge and capacity to adopt preventive measures, such as using masks, seeking timely medical care, and avoiding harmful environments. Mining jobs often involve direct contact with pollutants, which worsen respiratory risks. The absence of adequate protective measures intensifies these vulnerabilities. Contaminated water sources can indirectly impact respiratory health by weakening overall immunity or through secondary infections that affect the respiratory system. Higher-income households are better equipped to afford safer living conditions, healthcare, and nutrition, reducing their susceptibility to illnesses.

Marginal effects on determinants of Respiratory illness (All villages)

The marginal effects from the probit model reveal several key determinants of respiratory illness in the study villages. Education has a significant negative effect at 1 per cent level (β = -0.028; p = 0.000), indicating that individuals with higher education levels are less likely to suffer from respiratory illness. This implies that education promotes better hygiene, preventive healthcare behaviour, and awareness of

TABLE 8

Estimation of probit model on determinants of Respiratory illness (All villages)

Variables	Parameters	Co-efficient	Standard Error	P value
Gender	β_1	-0.433	0.790	2.27
Age	β_2	0.022	0.034	0.52
Education	β_3	-0.634	0. 0212	0.003
Mine job	β_4	2.147	0.916	0.019
water quality	β_5	-1.700	0.648	0.009
Household size	β_6	0.396	0.327	0.225
Income	β_7	-1.159	0.529	0.028
Intercept	A	3.778	2.269	0.096
ROC value	0.95			

Table 9

Marginal effects on determinants of respiratory illness (All villages)

Variables	Parameters	Marginal effects	Standard Error	P value
Gender	β_1	-0.019	0.034	0.583
Age	β_2	0.001	0.001	0.515
Education	β_3	-0.028	0.007	0.000
Mine job	β_4	0.094	0.035	0.008
Water quality	β_5	-0.074	0.022	0.001
Household size	β_6	0.017	0.014	0.208
Ln (Income)	β_7	-0.051	0.021	0.017

pollution-related risks. Mining job is positively significant at the (1%) level (β = 0.094; p = 0.008), revealing that individuals engaged in mining-related work face a higher probability of respiratory illness due to constant exposure to dust, toxic gases, and particulate matter. This underscores the urgency of improving occupational health safeguards in mining areas.

Water quality shows a negative and significant effect at the 1% level (β = -0.074; p = 0.001), suggesting that better access to clean water helps reduce respiratory illness, likely by enhancing immunity and reducing co-morbid waterborne diseases. Income is also significantly negative at the 5% level (β = -0.051; p = 0.017), implying that higher-income households are less prone to respiratory issues due to improved living conditions, access to healthcare and the ability to afford preventive infrastructure. Meanwhile, gender (β = -0.019; p = 0.583), age (β = 0.001; p = 0.515), and household size (β = 0.017; p = 0.208) are statistically non-significant, showing no decisive impact.

The study clearly reveals that coal mining adversely affects both agricultural productivity and public health. Farmers in mining regions earn lower returns and face technical inefficiencies, primarily due to poor input use and environmental degradation. Health outcomes are worse, with higher cases of respiratory illness linked to mining-related jobs. Sustainable development in these areas demands urgent policy focus on improving farming practices, healthcare access, clean water, and worker safety. Addressing

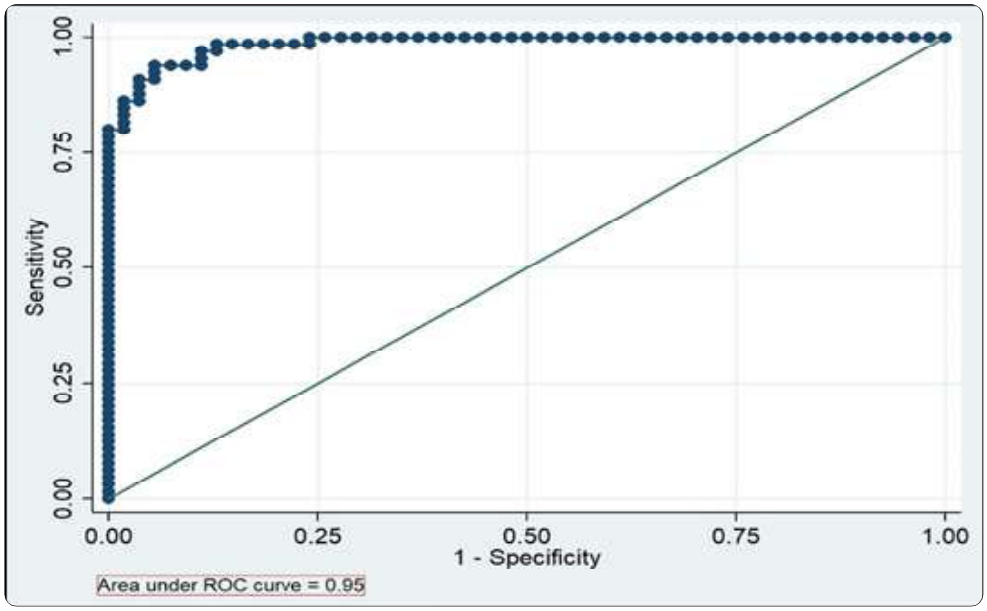


Fig. 2 : Receivers Operating curve for determinants of Respiratory Illness

these challenges can enhance agricultural efficiency and safeguard community well-being in mining-affected regions.

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Molecular Characterisation and Detection of Cucumber Green Mottle Mosaic Virus Infecting Cucumber by Loop Mediated Isothermal Amplification

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Abstract

Cucumber green mottle mosaic virus (CGMMV), a highly transmissible seed-borne *Tobamovirus*, poses a serious threat to cucurbit production worldwide. In the present study, leaf samples from symptomatic cucumber plants collected from Kolar, Karnataka, India and plant sap inoculated and mechanically to on to *Nicotiana glutinosa* and *Chenopodium giganteum* plants. The virus isolate was confirmed for CGMMV infection by RT-PCR amplification of the coat protein (CP) gene with specific primers with the expected amplicon of a ≈490 bp. Sequencing and phylogenetic analysis revealed that the current (KCG1) isolate (Acc. No. PX281938) formed a distinct lineage compared to other global isolates, underscoring regional genetic variability. To facilitate rapid diagnosis, a loop-mediated isothermal amplification (LAMP) assay targeting the CP gene was standardized, which produced the characteristic ladder-like banding pattern on 1.8 per cent agarose gel and enabled visual detection through a distinct hydroxynaphthol blue-mediated colour change from violet to sky blue. The integration of molecular characterization and LAMP assay provide a robust approach for precise identification of CGMMV and offers a practical, field-deployable diagnostic tool for seed health testing, quarantine inspection and large-scale epidemiological surveillance in cucurbit-growing regions.

Keywords : CGMMV, molecular-characterisation, LAMP, PCR

Cucumber (*Cucumis sativus* L.) is one of the most widely cultivated vegetables globally, belonging to the family, *Cucurbitaceae*, which also includes melons, pumpkins and gourds. It is one among the oldest cultivated crops, with historical records tracing its use back to over 3,000 years in regions of South Asia and later spreading to the Middle East, Europe and beyond (Mallik *et al.,* 2013). Botanically, cucumber is an annual climbing plant with elongated fruits composed mainly of water (over 90%), making it highly valued for hydration, low caloric content and refreshing texture (Sharma *et al.,* 2020).

Cucumber is severely affected by several viral pathogens that cause major yield and quality losses worldwide. The most common viruses include cucumber mosaic virus (CMV), which induces mosaic, chlorosis and leaf malformations and has been confirmed molecularly in multiple regions including Turkey (Guller *et al*., 2024); cucumber green mottle mosaic virus (CGMMV), a *Tobamovirus* causing vein chlorosis, fruit malformation and severe yield reduction, with resistance sources identified only in wild *Cucumis* species (Crespo *et al*., 2018) and zucchini yellow mosaic virus (ZYMV), papaya ringspot virus (PRSV), and watermelon mosaic virus (WMV-2), which frequently occur in mixed infections and produce severe mosaic, vein yellowing and fruit deformation (Cho *et al*., 2011). These viruses belong

to diverse families, including *Cucumovirus*, *Potyvirus* and *Tobamovirus* and their management is challenging due to mixed infections and rapid viral evolution (Martin-Hernandez and Pico, 2020).

CGMMV is a highly contagious and economically significant plant virus belonging to the genus, *Tobamovirus*. First identified in the United Kingdom in 1935, CGMMV has since spread globally, severely affecting cucurbit crops such as cucumber, watermelon, muskmelon and bottle gourd. The virus is seed-borne and mechanically transmitted, making it particularly difficult to eradicate once introduced into cropping systems. Its virions are rigid, rod-shaped particles (~300 × 15 nm), encapsulating a positive-sense single-stranded RNA genome of about 6.4 kb (Mandal *et al*., 2008).

CGMMV infection leads to characteristic symptoms such as systemic green mottle, mosaic patterns, leaf distortion, and in some cases, severe fruit disorders like pulp deterioration ('blood flesh' disease) in watermelon (Mandal *et al.,* 2008). The virus has shown remarkable stability in seeds and plant debris, enabling long-distance dissemination through contaminated seed lots and farming practices. Recent studies have also revealed its ability to infect alternative hosts, including weeds, which may act as reservoirs and complicate control strategies (Lovelock *et al*., 2023).

Molecular characterization of CGMMV has been essential in unravelling its genetic structure, diversity, and evolutionary dynamics. The viral genome encodes four open reading frames (ORFs), including a replication-associated protein (126 kDa), a read-through polymerase (183 kDa), a movement protein (MP) and a coat protein (CP) (Mandal *et al*., 2008). Comparative genomic studies have revealed low but significant variability among global isolates, with distinct phylogenetic clustering linked to geographical origins (Yeesarapat *et al*., 2013). Such molecular insights are crucial not only for taxonomy but also for designing precise diagnostic tools and breeding resistant cultivars. The present study emphasizes the molecular characterization of CGMMV, providing valuable insights into its genetic diversity and contributing to a deeper understanding of the distribution and prevalence of specific isolates within the regions.

Material and Methods

Source and Maintenance of the Virus Isolate

Leaf samples from cucumber plants showing typical CGMMV symptoms were collected from naturally infected fields of Kolar district, Karnataka, India. The samples were ground in a chilled mortar and pestle using 0.05 M potassium phosphate buffer (pH 7.0) supplemented with 0.02 per cent 2-β-mercaptoethanol, at a ratio of 2 mL buffer per gram of tissue. The crude sap was then filtered through double-layered muslin cloth to eliminate debris. For mechanical inoculation, celite powder (600 mesh, 0.025 g/mL sap) was added as an abrasive and the prepared inoculum was applied to the upper surface of *Nicotiana glutinosa* and *Chenopodium giganteum* leaves by gently rubbing with absorbent cotton in a uni-direction. Excess inoculum on the leaves was rinsed away with distilled water after ten minutes (Vinaykumar *et al*., 2018). Inoculated plants were kept in glasshouse to monitor symptom manifestation. The virus culture designated as KCG1 isolate and was maintained in the glasshouse at Department of Plant Pathology, UAS, GKVK, Bengaluru for further studies.

RNA Isolation and PCR Amplification

RNA was extracted from the leaf samples harvested from KCG-1 isolate inoculated on to tobacco plants maintained in the glasshouse, along with healthy leaf samples, using a modified phenol–chloroform and lithium chloride (LiCl) protocol as described by Netla *et al.* (2025). The integrity of the RNA was verified on a 1.2 per cent agarose gel, while quantification was performed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA). For cDNA synthesis, reverse transcription was carried out with a CGMMV CP- R primer. Briefly, 5 µg of total RNA was mixed with 10 pmol/µL of the primer and denatured at 70°C for 5 minutes. The reaction mixture was then supplemented with 4 µL of 5× RT buffer,

2.5 µL of 10 mM dNTP mix, 4 µL of DEPC-treated water and 0.5 µL of PrimeScript™ Reverse Transcriptase (200 U/µL), making up a final volume of 20 µL. Reverse transcription was performed at 42°C for 60 minutes, followed by enzyme inactivation at 70°C for 10 minutes (Khatib *et al*., 2025) followed by PCR amplification of full length coat protein gene of CGMMV.

CGMMV CP-specific primers were used for the PCR amplification and Table 1 lists the primer sequences, cycling conditions and expected amplicon size for PCR reaction. The 20 µL PCR reaction mixture consists of 10 µL of 2× PCR Master Mix (Emerald Amp, TaKaRa, Japan), 5 µL of nuclease-free water and 1.5 µL (10 pmol/µL) of both forward and reverse primers. Amplification was performed in a Proflex thermal cycler (Carlsbad, California, USA) along with negative control. A negative control in PCR is a reaction containing all components except the template DNA, used to ensure that any amplification observed is not due to contamination. The PCR products were electrophoresed on a 1.5 per cent agarose gel, stained with ethidium bromide and bands were visualised undera gel documentation system. The amplified products were Sanger sequenced at Barcode Bioscience Private Limited, Bengaluru.

Phylogenetic Analysis

The CGMMV CP sequence obtained by anger sequencing was subjected to BLASTn search and similar sequences were identified in the National Centre for Biotechnology Information (NCBI) database, Maryland, United States. The sequences showing similarity were retrieved from NCBI (Table 2) and was employed in phylogenetic analysis. Phylogenetic analysis was performed using MEGAXI software with 1000 bootstrap replication value (Tamura *et al*., 2021). This similarity index was represented in colour code matrix using SDTv1.3 software.

Loop-Mediated Isothermal Amplification

LAMP assays were carried out in a 25 µL reaction volume consisting of 2 µLcDNA template reverse transcribed using B3 primer (50 ng/µL), 1 µL each of outer primers F3 and B3 (10 µM), 2 µL each of inner primers FIP and BIP (10 µM), as well as loop primers LF and LB (10 µM). The mixture also contained 1.5 µL of 10 mM dNTPs, 1 µL of 5 M betaine, 6.5 µL nuclease-free water, 2.5 µL of 1X ThermoPol reaction buffer (20 mM Tris–HCl, 10 mM $(NH_4)_2SO_4$, 10 mMKCl, 2 mM$MgSO_4$ and 0.1% Triton X-100, pH 8.8 at 25 °C), 0.5 µL of 100 mM$MgSO_4$ and 1 µL of Bst DNA polymerase (8 U; New England Biolabs, USA). Amplification was performed in a water bath at 60 °C for 60 min, followed by heat inactivation at 80 °C for 10 min. The amplification products were resolved on 1.8 per cent agarose gel, electrophoresed at 65 V for 3 h, stained with ethidium bromide (10 mg/mL) and visualized

Table 1

List of primers designed and used in this study

Primer name	Primer sequence	Target region	Amplicon length
CGMMV CP-F	GACGGAATTCATGGCTTACAATCCGATCAC	Coat	≈490 bp
CGMMV CP-R	GCAGTCGACGCTTTCGAGGTGGTAGCCT	protein	
CGMMV - F3	TCACAAGGTACCGCTTTCC	gene	
CGMMV - B3	CGTATCCGTGGAGCTGAGA		
CGMMV - FIP	ACGACAGACGAGGGTAACGCA-AGACTCAAGCGGGAAGAGA		
CGMMV - BIP	TCTAGGTTCCCAGATGCGGGT-AGCGAAACGAAGATAGGCC		
CGMMV - LF	GACAGGGACTCGCGGAAA		
CGMMV – LB	CGCTTTCCTCAACGGTCCT		

TABLE 2

List of nucleotide sequences of different CGMMV isolates used in phylogenetic analysis along with source, country and identity per centage

Accession number	Virus	Source	Country	Identity score (%)
OQ198378.1	CGMMV	Hive collected pollen	Australia	96.30
KX353557.1	CGMMV	Squash	China	96.30
OQ198386.1	CGMMV	Hive collected pollen	Australia	96.30
OQ198385.1	CGMMV	Hive collected pollen	Australia	96.30
MH271441.1	CGMMV	Tobacco	Canada	96.26
DQ767636.1	CGMMV	Bottle gourd	India	96.26
AJ748352.1	CGMMV	Calabash	India	96.51
KM873787.1	CGMMV	Watermelon	China	96.42
KJ754195.1	CGMMV	Cucumber	China	95.90
OK338441.1	CGMMV	Pea	China	96.71
MH129050.1	CGMMV	Muskmelon	Thailand	96.18
KC852074.1	CGMMV	Tobacco	China	96.51
HG970177.1	CGMMV	Muskmelon	China	96.51
KX353559.1	CGMMV	Cucumber	Spain	97.74
MF510469.1	CGMMV	Cucumber	Canada	97.74
KJ729038.1	CGMMV	Bottle gourd	India	96.92
MF510467.1	CGMMV	Cucumber	Canada	97.74
MH426842.1	CGMMV	Cucumber	Canada	97.54
AJ429090.1	CGMMV	Cucumber	Germany	94.50
AB369274.1	CGMMV	Watermelon	South Korea	97.54
MW732125.1	CGMMV	Squash	Pakistan	96.51
MW732124.1	CGMMV	Bitter gourd	Pakistan	96.42
OR482969.1	CGMMV	Bitter gourd	Pakistan	96.42
EU366912.1	CGMMV	Cucumber	India	97.33
MN551284.1	CGMMV	Muskmelon	Korea	97.13
AJ459421.1	CGMMV	-	Korea	96.51
PX281938	CGMMV	Cucumber	India	-
L35073.1	Tobacco mosaic virus	Pepper	Korea	62.36
JF8103439.1	Tomato mosaic virus	Tomato	Spain	78.20

using a Gel Doc XR system (Bio-Rad, USA). For colorimetric detection, 0.2 µL of 20 mM hydroxynaphthol blue (HNB; Sigma-Aldrich, USA) was added to the reaction mixture before amplification. A positive LAMP reaction was indicated by a visible color change from violet to sky blue (Netla *et al*., 2025).

RESULTS AND DISCUSSION

Source and Maintenance of the Virus Isolate

The cucumber leaves sample showing typical CGMMV symptoms, such as mosaic and mottling symptoms on leaves, including light and dark green patches, yellowing, vein chlorosis and wrinkling (Ling

et al., 2014) were collected from the fields of Kolar district, Karnataka during February of 2024 followed by mechanical inoculation on to *N. glutinosa* and *C. giganteum*. Upon mechanical inoculation, *N. glutinosa* exhibited the local lesions and *C. giganteum* exhibited the mosaic symptoms. *Tobamoviruse* typically induce necrotic local lesions on *N.glutinosa*, which appear as small, dark brown to black spots within four days of inoculation (Plate 1). These lesions are a classic hypersensitive response, restricting viral spread to localized areas (Zhang *et al.,* 2008). *Chenopodium* spp. upon inoculation with tobamoviruses (including *C. giganteum*, similar to *C. amaranticolor* and *C. murale*, which are widely used as indicator plants), produces chlorotic lesions that are distinct, round and well-delimited, sometimes expanding into chlorotic spots or vein clearing, which depends on the virus strain (Kim *et al.,* 2002). The virus isolate was designated as KCG1 isolate (Fig. 1).

PCR Amplification

PCR amplification from total RNA extracted from symptomatic *N.glutinosa* leaf samples using CGMMV coat protein (CP)-specific primers successfully resulted in an expected amplicon of approximately 490 bp (Plate 2). No amplification was observed in healthy plant controls, confirming primer specificity. The results confirm the presence of CGMMV in the

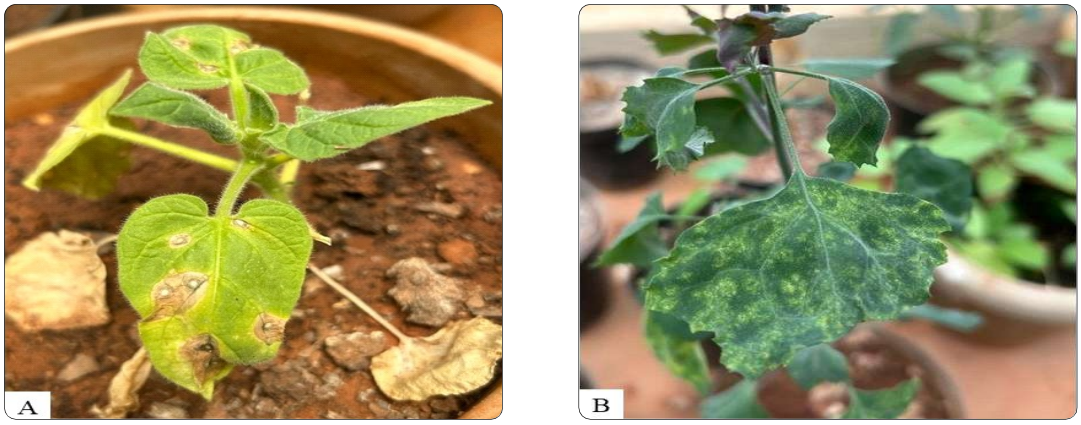

Plate 1 : Symptoms induced by CGMMV on A) *Nicotiana glutinosa* after 4 days of inoculation by infected cucumber leaf. B*) Chenopodium giganteum* after 7-8 days of inoculation by infected cucumber leaf

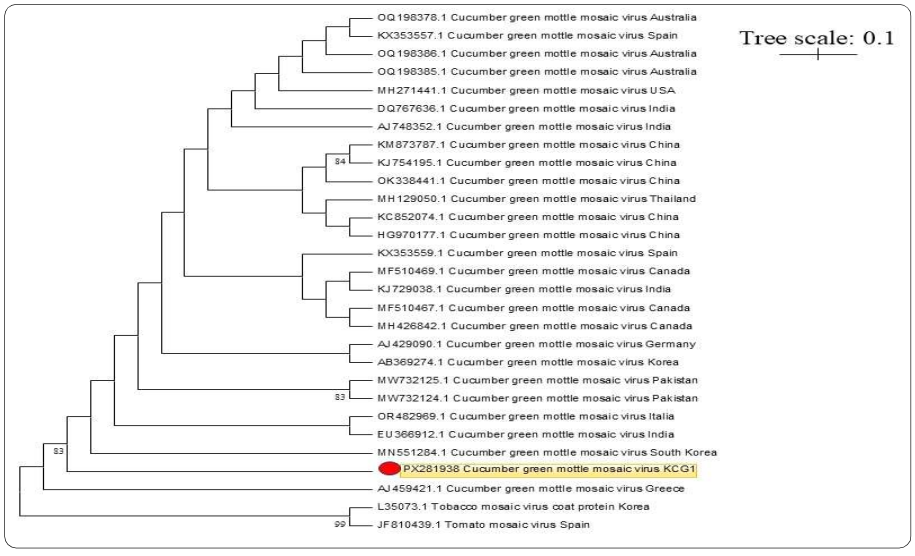

Fig. 1 : Phylogenetic tree based on the nucleotide sequence of CGMMV-KCG1isolate with the retrieved sequences from the NCBI database using MEGAXI software with 1000 bootstrap replication value

N. glutinosa plants inoculated with suspected CGMMV isolate collected from the cucumber fields confirming the virus isolate as CGMMV. Similar attempts in detecting CGMMV was reported earlier, where the CP gene region was amplified using the specific primers with expected amplicon length of the 480-500 bp range (Ling *et al.,* 2014 and Mandal *et al.,* 2008). These findings highlight the robustness of CP-based PCR assays for routine detection, given that the coat protein is highly conserved among CGMMV isolates. Similar results have been reported in diagnostic studies across different geographical regions, reinforcing the value of CP gene amplification in confirming field infections and supporting downstream applications such as sequencing, phylogenetic analysis and strain differentiation (Orfanidou *et al*., 2019).

Molecular Characterisation and Phylogenetic Analysis

The nucleotide sequence of ≈490 bp was obtained from the PCR amplified products using CGMMV coat protein specific primers. The obtained sequence was compared with the other twenty-six coat protein gene sequence of CGMMV along with coat protein gene of tobacco mosaic virus and tomato mosaic virus as out group (Table 2). The Phylogenetic tree was constructed using the MEGAXI software (Tamura *et al*., 2021). The phylogenetic analysis of coat protein (CP) gene sequences demonstrated that the CGMMV isolate PX281938 (KCG1) is distinct from the majority of other global isolates. Unlike the Indian, Chinese, Canadian and European isolates that formed well-supported clusters, KCG1 branched separately, indicating a more divergent lineage. This suggests that KCG1 isolate has undergone independent evolutionary changes, possibly influenced by host adaptation or regional selective pressures.

Although distantly related to the main Asian and European clades, KCG1 isolate still groups within the CGMMV complex, confirming its identity as a variant of the same virus species. Its separation from closely related isolates such as those from India (DQ767636.1, AJ748352.1, EU366912.1) and China (HG970177.1, KC852074.1) highlights the genetic diversity of

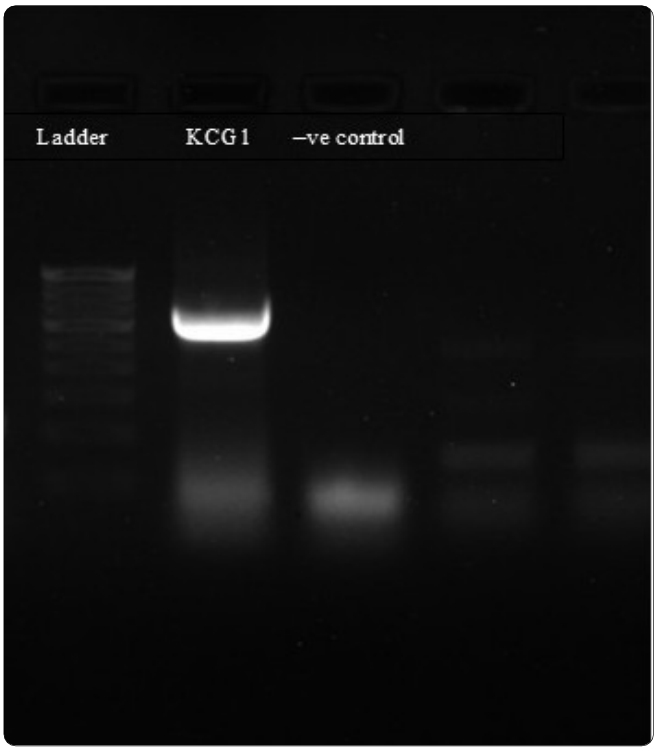

Plate 2 : PCR amplification of CGMMV coat protein gene on agarose gel electrophoresis

CGMMV isolates and suggests limited gene flow between KCG1 isolates and these populations. Interestingly, the basal positioning of KCG1 isolate near the outgroups (*Tobacco mosaic virus* and *Tomato mosaic virus*) may reflect early divergence or reduced similarity compared to other CGMMV isolates (Fig.1). This is represented in colour code matrix using SDTv1.3 software (Fig. 2).

Loop Mediated Isothermal Amplification

The LAMP assay developed successfully amplified the target coat protein (CP) gene of CGMMV. The amplified products, when resolved on 1.8 per cent agarose gel electrophoresis, exhibited a characteristic ladder-like banding pattern, confirming successful amplification of the viral genome (Plate 3). No amplification was observed in the negative controls and healthy *N. glutinosa* samples, indicating the assay's specificity. In addition, positive reactions were visually confirmed through a distinct colour change in the reaction mixture, enabling rapid detection without the need for electrophoresis. The absence of amplification in negative control ensures no contamination by showing no amplification without template DNA, while a healthy control confirms assay specificity by showing no amplification using nucleic acid from a pathogen-free plant. The visible colour change provided a rapid, user-friendly diagnostic option (Muttappagol *et al.,* 2023). These findings are consistent with earlier reports demonstrating the efficiency of LAMP for plant virus detection, where high sensitivity and rapid amplification were achieved under isothermal conditions (Notomi *et al*., 2000 and Zhao *et al*., 2019). LAMP's isothermal nature, rapid turnaround and potential for on-site use provide major advantages (Mori & Notomi, 2009 and Niessen, 2015).

CGMMVis a seed-borne *Tobamovirus* that causes severe yield losses in cucurbits. In this study, leaf samples from symptomatic cucumber plants from Kolar, Karnataka, India, were collected, mechanically inoculated on to *N. glutinosa* and *C. giganteum* confirmed for the presence of CGMMV through RT-PCR using primers specific to coat protein (CP) gene. Sequencing and phylogenetic analysis revealed that the isolate PX281938 (KCG1) in the current study formed a distinct lineage compared to global isolates, indicating regional genetic divergence. To enable rapid detection, a loop-mediated isothermal amplification (LAMP) assay was developed targeting the CP gene. The assay produced a characteristic ladder-like banding pattern on agarose gel and allowed visual

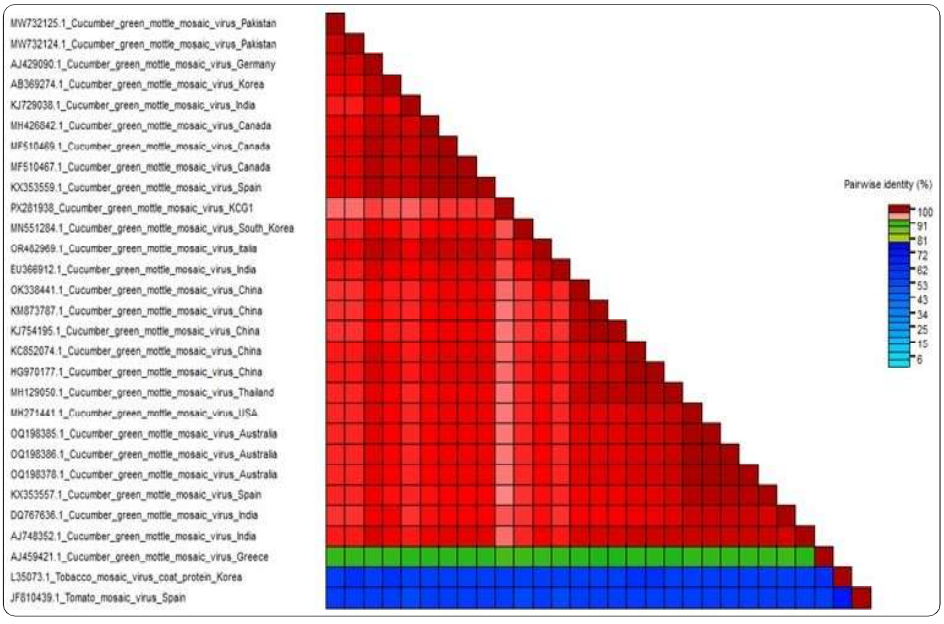

Fig. 2 : Graphical representation of percentage pairwise genomic scores and nucleotide identity plot for coat protein gene of CGMMV-KCG1isolate using SDTv1.3

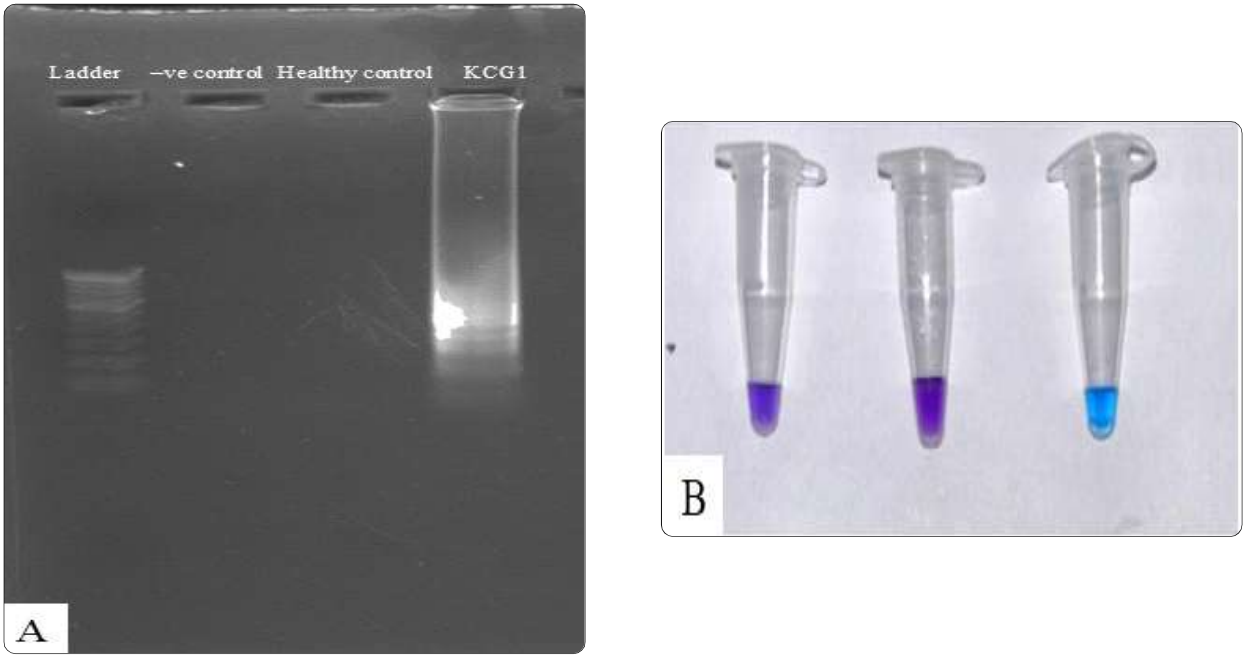

Plate 3 : Detection of CGMMV by Loop mediated isothermal amplification. A) Visualisation of LAMP amplified product on 1.8 per cent agarose gel electrophoresis using ethidium bromide dye. B) Visual detection of amplified LAMP product using hydroxynaphthol blue

detection by a colour change from violet to sky blue. This combined molecular characterization and sensitive diagnostic tool provides valuable support for seed health testing, quarantine inspections and surveillance strategies against CGMMV.

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Assessment of Ground Water Quality Under Different Land Use System in Post Gold Mining Areas of Kolar District, Karnataka

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V. Ramamurthy &
B. Tambat :
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Abstract

Underground gold mining at Kolar Gold Fields (KGF), Karnataka was carried out for more than a century, which disturbed the groundwater aquifers. In addition, leaching from mill tailings dumps has also been contaminating groundwater in this area. The aim of the study was to assess the groundwater quality and its suitability for irrigation purpose. This study assessed the groundwater quality in different land use systems agriculture, horticulture, barren and forest around KGF to evaluate its suitability for irrigation purposes. It was found that the pH of groundwater samples ranged between 5.52 to 7.81, indicating slightly acidic to neutral conditions. Cation concentrations followed the order $Na^+ > Ca^{2+} > Mg^{2+} > K^+$, with sodium being dominant, while bicarbonates were the major anions ($HCO_3^- > Cl^- > SO_4^{2-} > CO_3^{2-}$). Electrical conductivity (EC) values varied between 198 and 2156 µS cm^{-1}, with 25 per cent samples exceeding WHO limits, largely due to fertilizer application, mining effluents and sewage inflows. Salinity hazard analysis revealed that 72 per cent of samples fell under high salinity (C3), posing risks to sensitive crops. However, sodium absorption ratio (SAR) values placed all samples under the excellent category (S1). Residual sodium carbonate (RSC) values indicated that 13-53 per cent of samples across land use systems fell under high alkalinity, suggesting risks of sodium accumulation in soils. Asper Kelly's index 68 per cent of samples were found to be suitable for irrigation, while the permeability index (PI) indicated good groundwater efficiency for irrigation (25–75%). Overall, the study concludes that groundwater in the KGF region remains moderately suitable for irrigation, though localized risks of salinity, alkalinity and heavy metal contamination persist.

Keywords : Ground water aquifers, Heavy metals, Kolar gold fields, Land use systems, Water quality Mining dumps

Rapid urbanization and industrialization have led to large amounts of toxic contaminants being released into the environment worldwide. Some of these contaminants occur naturally, but anthropogenic sources, especially mining activities, have contributed significantly to their increase. However, developing countries like India, due to lack of high-profile industrial techniques and equipment, eluting effluents from the industrial process may contain various hazardous substances which greatly affect the environmental and human health (Ramappa and Munniswamy, 2017). Large quantities of waste are produced during mining activity, particularly in gold mines which release over 99 per cent of extracted ore as waste to the environment mainly as tailings primarily consist of sand, silt and clay sized materials with chemicals and process water.

The tailings contain many heavy metals, some of which are toxic contaminants in the water bodies that

may cause adverse effect on the ecosystem around the metal mines. Therefore, contamination of water bodies by heavy metals is a primary concern in the mining sites because of their toxicity, persistence. The continuous impacts of mining activities are land erosion and water pollution (Kinimo *et al*., 2018). Enormous solid wastes with high risks to acid generation and heavy metal leaching may cause contamination of surface water and groundwater during mining operation or even long after mine closure (Shu *et al*., 2001). Consequently, AMD significantly impacts water quality and natural ecosystems (Hu, 1998).

The Kolar Gold Fields (KGF) mines are seated towards the southern end of a narrow strip of schist in the Kolar district of Karnataka. At KGF, gold has been mined to a depth of 3 km below the surface with 650 km of tunnel work and mining was carried out over 120 years and about 40 million tonnes of mill tailings were accumulated. The Kolar Gold Fields, once a major centre of gold mining activity, have left behind large volumes of mine tailings and acid mine drainage (AMD), which continue to act as long-term sources of groundwater contamination. The percolation of acidic effluents and leachates enriched with dissolved metals from these mine residues has significantly affected the quality of groundwater in the surrounding areas. Groundwater, being the primary source of drinking water and irrigation in the Kolar District, is highly susceptible to such contamination, resulting in potential health risks and limitations for its safe utilization. Even decades after the cessation of mining operations, the legacy of AMD and tailings continues to impair the hydro chemical characteristics of aquifers, raising concerns regarding the sustainability of water resources in this region. In view of this, a detailed study was undertaken with the objective of assessing the quality of groundwater with a hypothesis weather it is suitable for irrigation or not, under different land use systems in the post-mining areas of Kolar District, Karnataka.

Material and Methods

Sampling Locations

Kolar Gold Fields (KGF) is a significant historical and mining region located in the KGF taluk of Kolar district in Karnataka, India. Positioned at approximately 12.9617 °N latitude and 78.2707 °E longitude (Fig. 1). A detailed survey was carried out within KGF to identify ground water resources in different land use systems of KGF. Four land use systems agriculture land use system (ALUS),

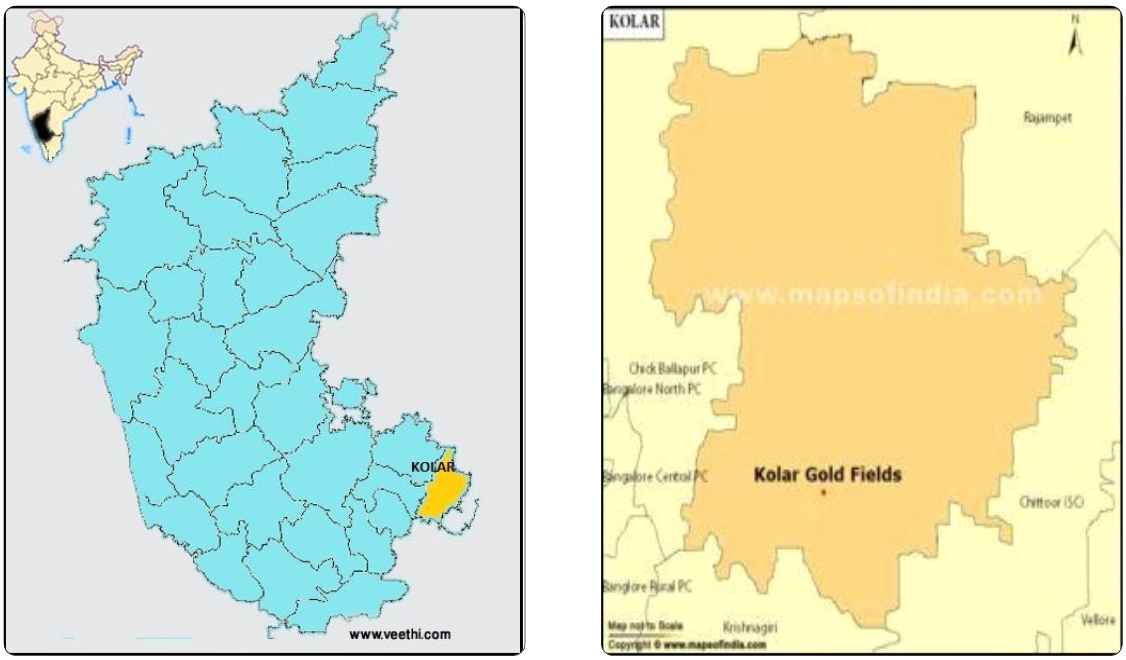

Fig. 1 : Kolar District map

horticulture land use system (HLUS), barren land use system (BLUS) and forest land use system (FLUS) were identified for collecting ground water samples Source (Roy *et al.,* 2012).

Collection of Water Samples

Water samples (groundwater) were collected from different sources *viz.,* borewells, dug wells and water bodies along the different land use systems of Kolar gold fields. Fifteen (15) groundwater samples (borewell) in each land use system agriculture land use system (ALUS), horticulture land use system (HLUS), barren land use system (BLUS) and forest land use system (FLUS) were collected. Total sixty (60) ground water samples were collected from all the land use systems and coordinates data are mentioned in Table 1. Water samples were collected in clean polythene bottles. The samples were collected in different seasons like post-monsoon (October-November). The samples collected were brought to the laboratory at UAS, GKVK, Bengaluru for detailed analysis. pH and EC were estimated by potentiometric and conductometric methods as proposed by (Jackson, 1973). Sodium and potassium were measured by flame photometry (Jackson, 1973). Calcium and magnesium were determined with Versenate titration method (Page *et al*., 1982). Carbonates, bicarbonates and chlorides were estimated by titration method (Jackson, 1973). Sulphates were estimated through turbidometric method (Black, 1965). Heavy metals (Pb, Cd, Ni and As) were estimated by atomic absorption spectrophotometry by (Lindsay and Norwell, 1978). Sodium Adsorption Ratio (SAR), Residual Sodium Bicarbonates (RSC), Kelly's ratio (KR), Permeability index (PI) and per cent sodium were computed using standard formulae:

Sodicity Hazard: Sodium Adsorption Ratio (SAR)

SAR values of the water samples were calculated as follows and all ionic concentrations are expressed in mg L^{-1} (Sposito and Mattigod, 1977).

$$SAR = \frac{Na^{+}}{\sqrt{\frac{Ca^{+2} + Mg^{+2}}{2}}}$$

Table 1

Coordinates of ground water samples of different land use systems

Agriculture land use system		Horticulture land use system		Barren land use system		Forest land use system	
Latitude (N) (Degree decimals)	Longitude (E) (Degree decimals)	Latitude (N) (Degree decimals)	Longitude (E) (Degree decimals)	Latitude (N) (Degree decimals)	Longitude (E) (Degree decimals)	Latitude (N) (Degree decimals)	Longitude (E) (Degree decimals)
12° 28' 45''	78° 30' 41''	12° 98' 62''	78° 26' 61''	12° 28' 25''	78° 30' 44''	12° 98' 61''	78° 30' 65''
12° 31' 56''	78° 30' 56''	12° 98' 21''	78° 30' 64''	12° 28' 22''	78° 30' 07''	12°97' 43''	78° 28' 22''
12° 45' 41''	78° 30' 21''	12° 98' 54''	78° 30' 22''	12° 27' 26''	78°28' 54''	12°97' 31''	78° 28' 24''
12° 22' 18''	78° 28' 63''	12° 97' 12''	78° 28' 95''	12° 27' 72''	78° 28' 58''	12° 98' 54''	78° 28' 12''
12° 19' 56''	78° 28' 21''	12° 97' 12''	78° 28' 25''	12° 45' 85''	78°27' 57''	12° 97' 64''	78° 24' 45''
12° 21' 41''	78° 28' 45''	12° 98' 15''	78° 27' 22''	12° 31' 53''	78° 27' 60''	12° 98' 34''	78° 52' 25''
12° 21' 53''	78° 27' 24''	12° 98' 56''	78° 27' 21''	12° 62' 41''	78° 27' 65''	12° 97' 65''	78° 42' 41''
12° 22' 50''	78° 27' 62''	12° 97' 14''	78° 27' 12''	12° 52' 41''	78° 27' 64''	12° 97' 74''	78° 56' 38''
12° 11' 13''	78° 27' 71''	12° 97' 45''	78° 27' 23''	12° 55' 48''	78°27' 47''	12° 98' 28''	78° 41' 74''
12° 21' 41''	78° 27' 56''	12° 97' 47''	78° 27' 24''	12° 41' 78''	78°26' 24''	12° 72' 34''	78° 45' 56''
12° 21' 53''	78° 27' 85''	12° 97' 58''	78° 26' 58''	13° 12' 54''	78° 26' 41''	12° 97' 56''	78° 95' 54''
13° 22' 18''	78° 26' 41''	13° 12' 54''	78° 26' 56''	13° 11' 65''	78° 26' 45''	12° 21' 78''	78° 56' 47''
13° 19' 56''	78° 26' 89''	13° 12' 58''	78° 26' 06''	12° 41' 31''	78° 27' 65''	12° 98' 34''	78° 54' 56''
12° 21' 41''	78° 26' 42''	12° 98' 47''	78° 26' 14''	12° 23' 76''	78° 26' 87''	12° 98' 89''	78° 94' 47''
12° 21' 39''	78° 26' 87''	12° 98' 89''	78° 26' 18''	12°25' 89''	78° 30' 12''	12° 97' 25''	78° 54' 21''

Alkalinity Index : Residual sodium carbonate (RSC)

RSC values of the water samples were calculated after the analysis of the samples for different chemical constituents as follows (Eaton, 1950).

$RSC = (CO_3^{-2} + HCO_3^{-}) - (Ca^{+2} + Mg^{+2})$

Kelly's Ratio/ Index : KI was calculated using the equation given below.

$$KI = \frac{Na^{+}}{Ca^{+2} + Mg^{+2}}$$

Permeability Index (PI):

PI of water samples were calculated using the following formula.

$$PI = \frac{Na^{+} + \sqrt{HCO_3^{-}}}{(Ca^{+2} + Mg^{+2} + Na^{+})} \times 100$$

Per cent sodium

$$Na\ (\%) = \frac{Na^{+}}{(Ca^{+2} + Mg^{+2} + Na^{+} + K^{+})} \times 100$$

Results and Discussion

Water quality parameters like pH, EC, cations, anionic concentration and heavy metal contents of ground water are presented in Table 2. SAR, RSC, KI, PI and per cent Na are presented in Table 3 and Table 4.

pH

The pH of water samples in different land use systems in the range of 5.52 to 7.81, 5.65 to 7.76, 5.88 to 6.85 and 7.06 to 7.75 with an average value of 7.23, 7.10, 6.31 and 7.21 in agriculture, horticulture, barren and forest land use systems, respectively (Table 2) and (Fig. 2). The pH of groundwater in different land use systems are in the range of slightly acidic to neutral. Though the pH of most of the samples did not exceed the standards prescribed for drinking, irrigation as well as for livestock use, there is every possibility that the values would reach the undesirable limits in groundwater bodies (BIS, 2012), considering the changes in the intensity of cultivation practices extending into mining zone in the due course of time. This indicates that the sampled groundwater is slightly acidic to alkaline.

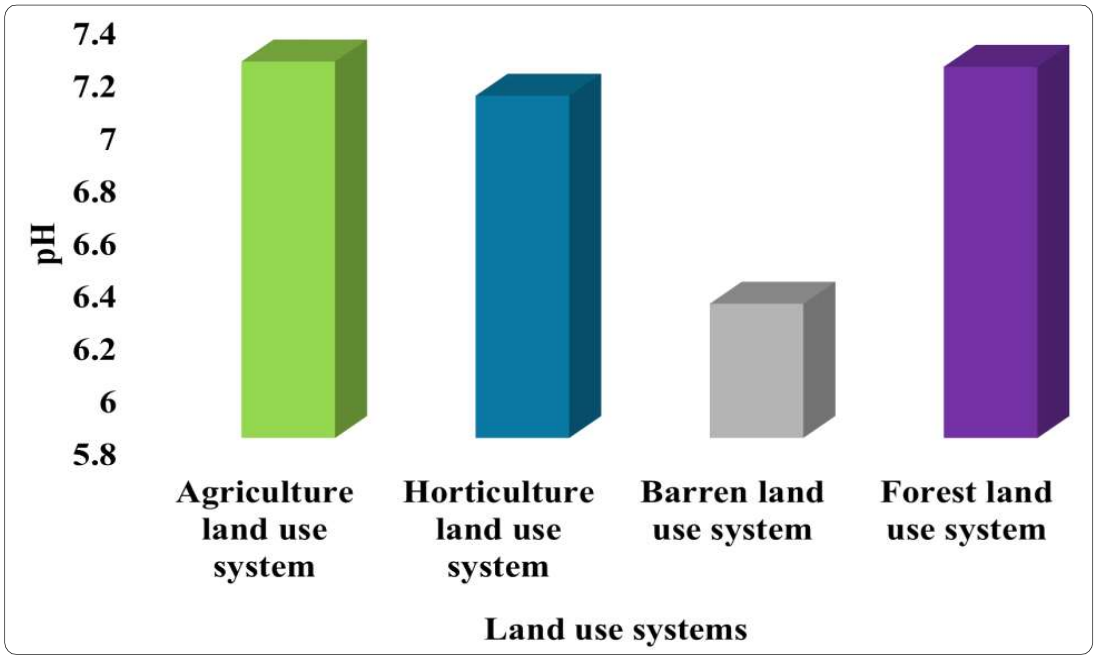

Fig. 2 : pH of ground water samples of different land use systems

TABLE 2

Descriptive statistics of chemical properties of ground water samples collected under different land use systems in post gold mining areas of Kolar district, Karnataka

Elements	Agriculture land use system (n=15)			Horticulture land use system (n=15)			Barren land use system (n=15)			Forest land use system (n=15)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
pH	5.52- 7.81	7.23	0.33	5.65-7.76	7.1	0.26	5.88-6.85	6.31	0.33	7.06-7.75	7.21	0.21
EC (µs cm^{-1})	245-2156	1353.26	572.70	205-2054	1384.20	536.58	325-1524	1160.73	326.78	198-1125	642.46	229.16
Ca (mg l^{-1})	33-138	88.67	34.66	41-119	74.30	30.44	19-73	49.34	15.03	22-75	42.93	17.08
Mg (mg l^{-1})	12-102	45.14	24.08	12-48	24.11	9.57	9-74	27.50	16.91	13-41	23.54	9.07
K (mg l^{-1})	4.85-12.06	7.98	2.72	2.33-7.17	4.93	1.66	1.63-7.21	4.20	1.86	1.12-7.10	4.40	1.95
Na (mg l^{-1})	68-179	132	29.08	42-165	97.82	38.00	30-154	74.90	39.57	24-146	90.00	40.04
CO_3^{-2}(mg l^{-1})	24-240	140.20	71.67	18-164	93.26	47.80	18-412	77.80	35.49	14-120	71.06	31.38
HCO_3^-(mg l^{-1})	183-658	353.93	145.46	112-540	261.80	137.59	69-412	247.06	111.12	101-256	172.46	55.14
Cl^- (mg l^{-1})	129-356	236.67	2.72	113-314	215.40	1.66	89-203	143.54	1.86	84-185	127.27	1.95
SO_4^{-2} (mg l^{-1})	125-248	179.75	29.08	68-229	156.78	38.00	88-173	131.10	39.57	55-141	100.78	40.04
Ni (mg l^{-1})	0.20-0.34	0.27	0.04	0.10-0.31	0.19	0.09	0.10-0.28	0.18	0.08	0.10-0.18	0.09	0.04
Cd (mg l^{-1})	0.04-0.08	0.05	0.11	0.04-0.05	0.04	0.09	0.02-0.05	0.03	0.08	0.03-0.06	0.04	0.04
Pb (mg l^{-1})	0.07-0.41	0.25	0.04	0.05-0.32	0.18	0.09	0.06-0.25	0.14	0.08	0.02-0.23	0.11	0.04
As (mg l^{-1})	0.02-0.04	0.03	0.11	0.01-0.03	0.02	0.09	0.01-0.04	0.02	0.08	0.01-0.02	0.02	0.05

TABLE 3

Ground water irrigation quality parameters under different land use systems of Kolar district, Karnataka

Quality Parameters	Agriculture land use system (n=15)		Horticulture land use system (n=15)		Barren land use system (n=15)		Forest land use system (n=15)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
SAR (mg l^{-1})	1.31-2.87	2.11	0.66-3.53	1.86	0.52-2.70	1.51	0.66-3.45	2.01
RSC (mg l^{-1})	0.14-4.95	2.28	0.02-4.65	1.67	0.02-6.25	2.08	0.17-2.78	1.08
KI	0.39-1.76	0.82	0.21-1.78	0.84	0.21-1.33	0.73	0.28-1.58	1.06
PI (%)	41.96-88.95	60.68	42.87-81.53	63.55	45.57-80.98	66.89	48.58-88.11	69.80
Na (%)	27.46-62.96	42.28	17.38-63.10	41.98	16.80-56.44	39.59	21.18-68.09	46.75

TABLE 4

One-way statistical analysis of ground water irrigation quality parameters under different land use systems of Kolar district, Karnataka

Quality parameters	Agriculture LUS ± SD (n=30)	Horticulture LUS ± SD (n=30)	Barren LUS± SD (n=30)	Forest LUS± SD (n=30)	Between land use systems	
					S.Em.	LSD* (p=0.05)
SAR (mg l^{-1})	2.11^{a} ± 0.59	1.86^{a} ± 0.81	1.51^{a} ± 0.68	2.01^{a} ±0.94	0.206	NS
RSC (mg l^{-1})	2.28^{a} ± 1.96	1.67^{a} ± 1.88	2.08^{a} ± 2.03	1.08^{a} ±0.91	0.470	NS
KI	0.82^{a} ± 0.39	0.84^{a} ± 0.46	0.73^{a} ± 0.32	1.06^{a} ±0.57	0.120	NS
PI (%)	60.68^{a} ± 12.75	63.55^{a} ± 10.91	66.89^{a} ± 11.94	69.80^{a} ±12.19	3.19	NS
Na (%)	42.28^{a} ± 10.41	41.98^{a} ± 12.37	39.59^{a} ± 11.17	46.75^{a} ±14.81	3.28	NS

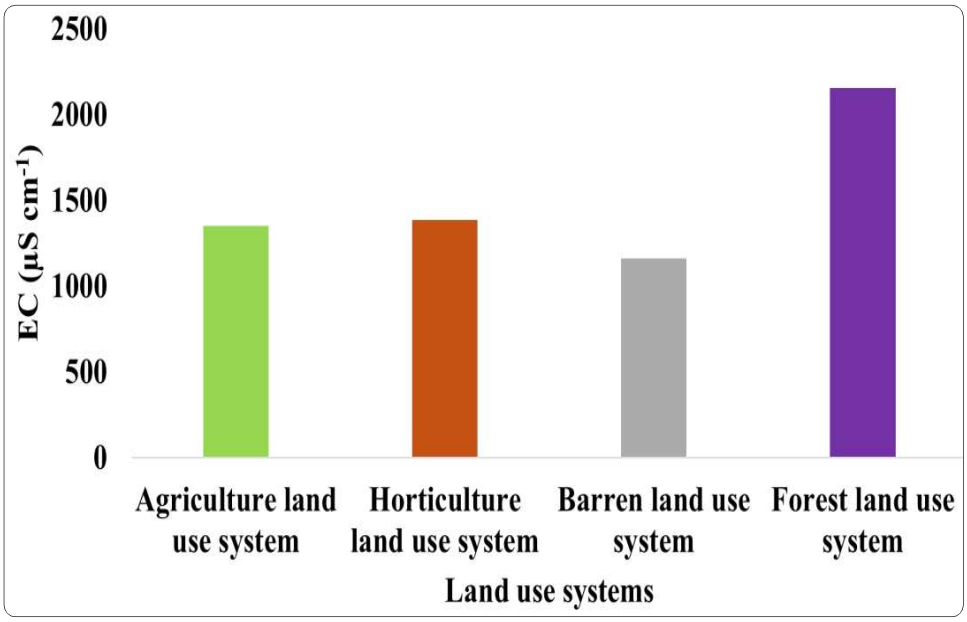

Fig. 3 : EC of ground water samples of different land use systems

Electrical Conductivity (µS cm^{-1})

Electrical conductivity indicates the amount of total dissolved salts present in water samples (Harilal *et al*., 2004). EC values were observed in the agriculture, horticulture, barren and forest land use systems were in the range of 245- 2156 µS cm^{-1}, 205- 2054 µS cm^{-1}, 325- 1524 µS cm^{-1} and 198 - 1125 µS cm^{-1}, respectively (Table 2) and (Fig 3). Higher values of EC were observed in agriculture land use system; this might be due to uses of fertilizers which dissolvesions and increases the EC and use of poor-quality irrigation water had resulted in accumulation of salts. Ground water samples recorded higher number of salts and it's attributed to the high degree of anthropogenic activities such as mining waste disposal, sewage inflow and agriculture runoff (Pandit, 2002). Significant increase in conductivity could be an indicator that a discharge or some other source of pollution has entered in a stream (Singh and Hussian, 2016). Electrical conductivity values are more influenced by physical (climate, hydrology) and chemical factors (local geology, mineral solubility) and by human impacts (use of fertilisers, changes in vegetation) compared to biological factors (Pedrosa and Rezende, 1999) and (Lekhashree *et al*., 2016).

Cation Concentration (Ca, Mg, K and Na)

The concentration of cation was in the ascending order of Na^{+}> Ca^{2+}> Mg^{2+}> K^{+} in groundwater of different land use systems in Kolar gold mining area. The sodium ion is dominant cation in the ground and surface water and its concentration in ground water ranged between 68 to 179, 42-165, 30-154 and 24 to 146 mg l^{-1} (Table 2) with a mean of 132, 97.82, 74.90 and 90 mg l^{-1} in agriculture, horticulture, barren and forest land use systems respectively (Fig. 4). The overall quality of ground water in the study area varied from 24 to 179 mg l^{-1} with a mean of 98.75 mg l^{-1}.

The second most dominant cation is calcium. The calcium concentration of the groundwater samples in the study area varied from 19 to 138mg l^{-1} with an average value of 63.81mg l^{-1} (Table 2). The range of Ca content in groundwater varied along agriculture, horticulture, barren and forest land use systems with a range of 33 to 138, 41 to 119,19 to 73 and 22-75 mg l^{-1} with mean of 88.67, 74.30, 49.34 and 42.93 mgl^{-1}, respectively. The higher calcium in ground water samples was due to calcium carbonate minerals deposited in the aquifer matrix (Gultekin, 1998).

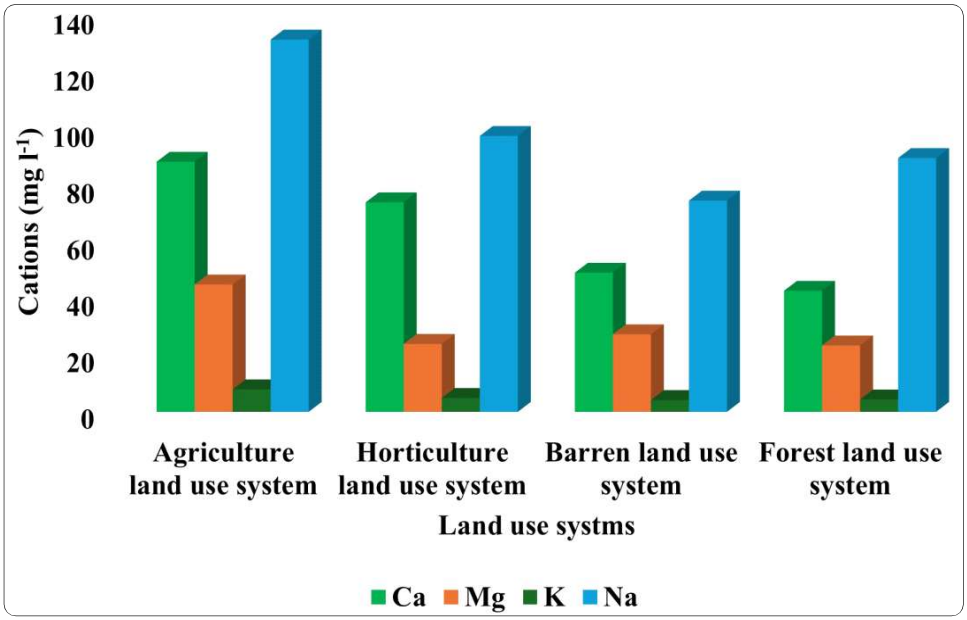

Fig. 4: Cationic concentration of ground water samples of different land use systems

The third most dominant cation is Mg. Compared to calcium, the Mg ion concentration in the samples were low, with a range of 9 to 102mg l^{-1} and an average of 30.07mg l^{-1} (Table 2) in groundwater samples. The magnesium concentration of the groundwater samples in the study area varied between 12 to 102, 12 to 48, 9 to 74 and 13 to 41mg l^{-1} (Table 2) with mean of 45.14, 24.11, 27.50 and 23.54mg l^{-1} (Fig. 5) in agriculture, horticulture, barren and forest land use systems, respectively.

Potassium is the fourth most dominant cation in the study area. The potassium concentration in the groundwater samples varied between 7.98, 4.93, 4.20 and 4.40mg l^{-1}, in agriculture, horticulture, barren and forest land use systems, respectively (Table 2). The range of potassium in groundwater varied between 4.85 to 12.06, 2.33 to 7.17, 1.63 to 7.21 and 1.12 to 7.10mg l^{-1} in agriculture, horticulture, barren and forest land use systems, respectively. In the overall groundwater samples, potassium content in the study area was within the range of 1.12 to 12.06mg l^{-1} with an average of 5.38mg l^{-1}.

Anions Concentration (CO_3^-, HCO_3^-, Cl^-, SO_4^{-2})

The order of the major anions according to their abundance in the study area is HCO_3^-> Cl^-> SO_4^{-2}> CO_3^{-2} with bicarbonates as the dominant anion in ground water samples of mining areas. Carbonate and bicarbonate ions, which are the main components of alkalinity, merged in soil with calcium or magnesium and precipitates as calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$). The concentration of CO_3^{-2} and HCO_3^- in the groundwater samples varied between 183 to 658, 112 to 540, 69 to 412 and 101 to 256mg l^{-1} and 24 to 240, 18 to 164, 18 to 412 and 14 to 120 mg l^{-1}, respectively with a mean of 353.93, 261.80, 247.06 and 172.46 mg l^{-1} and 140.20, 93.26, 77.80 and 71.06 mg l^{-1} in agriculture, horticulture, barren and forest land use systems, respectively. They were within the permissible limit according to (BIS, 2012).

Chloride is the second dominant anion in the study area. In the present study chloride concentration in groundwater ranged from 129 to 356, 113 to 314, 89 to 203 and 84 to 185mg l^{-1} with a mean value of 236.67, 215.40, 143.54 and 127.27mg l^{-1} in agriculture, horticulture, barren and forest land use systems, respectively (Table 2).

The concentration of sulphates in groundwater samples varied between 125 to 248, 68 to 229, 88 to 173 and 55 to 141mg l^{-1} with a mean of 179.75, 156.78, 131.10 and 100.78mg l^{-1} in agriculture, horticulture,

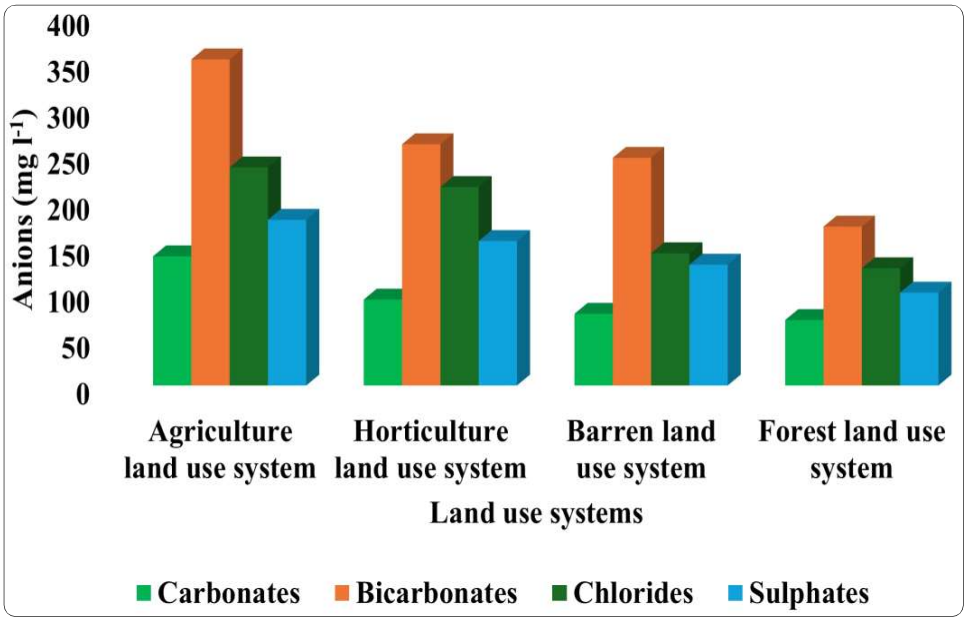

Fig. 5 : Anionic concentration of ground water samples of different land use systems

barren and forest land use systems, respectively (Table 2). The overall concentration of sulphates in the groundwater of the study area is within the range of 55 to 248mg l^{-1} with an average of 142.10mg l^{-1}. The sulphate concentrations of the maximum ground water samples were elevated but not higher than the maximum allowable limit of 200 mg l^{-1} (BIS, 2012).

Heavy Metal Concentration

The range of nickel in groundwater varied from 0.20 to 0.34 in agriculture, 0.10 to 0.31 in horticulture, 0.10 to 0.28 in barren and 0.10 to 0.18 mg l^{-1} in forest land use systems with an average value of 0.27, 0.19, 0.18 and 0.09mg l^{-1} in agriculture, horticulture, barren and forest land use systems, respectively (Table 2) and (Fig. 6). The cadmium content in groundwater varied with 0.04 to 0.08, 0.04 to 0.05, 0.02 to 0.05 and 0.03 to 0.06mg l^{-1} with mean of 0.05, 0.04, 0.03 and 0.04mg l^{-1} in agriculture, horticulture, barren and forest land use systems, respectively (Table 2). Lead concentration in groundwater varied with 0.07 to 0.41, 0.05 to 0.32, 0.06 to 0.25 and 0.02 to 0.23mg l^{-1} with mean of 0.25, 0.18, 0.14 and 0.11mg l^{-1}in agriculture, horticulture, barren and forest land use systems, respectively (Table 2). The concentration of arsenic in groundwater varied with 0.02 to 0.04, 0.01 to 0.03, 0.01 to 0.04 and 0.01 to 0.02mg l^{-1} with mean of 0.03, 0.02, 0.02 and 0.02mg l^{-1} in agriculture, horticulture, barren and forest land use systems, respectively (Fig. 6).

Agriculture land use system was found to be higher in heavy metal content when compared to all land use systems. Though heavy metal concentration is within the prescribed standards for drinking and irrigation uses, results indicated that they were fast increasing to alarming levels for all selected end uses in all land use systems. Gold ores frequently occur alongside sulphide minerals like pyrite, sphalerite and galena which exposed to air and water and generate sulphuric acid. This acid dissolves metals like As, Pb, Cu, Zn, Ni, Fe and Mn into water system (Baneh *et al*., 2024) and (Suchitra *et al*., 2011).(Chakraborti *et al*.,2013) in Kolar's greenstone belt (Mangalur region) report extremely elevated arsenic levels in groundwater.

Sodicity Hazard (Sodium Absorption Ration-SAR: mg l^{-1})

The calculated value of SAR in the groundwater ranged between 1.31 to 2.87, 0.66 to 3.53, 0.52 to 2.70 and 0.66 to 3.45mg l^{-1} (Table 3) with mean of 2.11, 1.86, 1.51 and 2.01mg l^{-1} (Table 4) in agriculture,

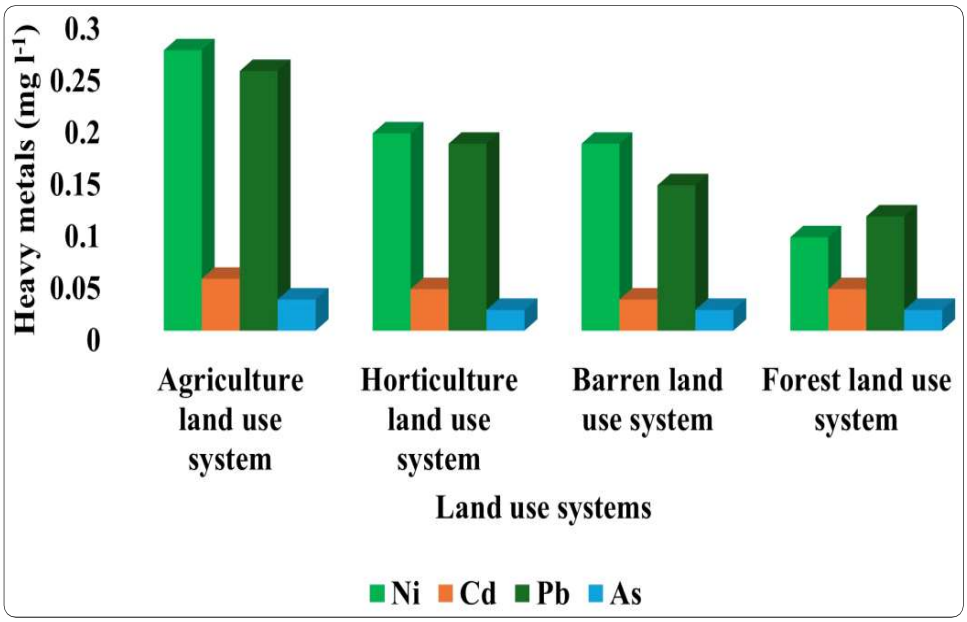

Fig. 6 : Heavy metal content of ground water samples of different land use systems

Table 5

Ground water quality for salinity hazard under different land use systems of Kolar district, Karnataka

Classes		Ranges ($\mu S\ cm^{-1}$)	No of samples (%)				Total
			ALUS	HLUS	BLUS	FLUS	
C1	Low	< 250	0 (0)	1 (7)	0 (0)	2 (13)	3 (5)
C2	Medium	250 - 750	1 (7)	1 (7)	1 (7)	11 (74)	14 (23)
C3	High	750 - 2250	14 (93)	13 (86)	14 (93)	2 (13)	43 (72)
C4	Very high	> 2250	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Total No. of samples					60 (100)

horticulture, barren and forest land use systems, respectively. All ground water samples were ideal or excellent (S1) in agriculture, horticulture, barren and forest land use systems (Eaton, 1950). In the study area, out of 60 samples all samples fall under class-S1 (Table 6).

Alkalinity Hazard/ Residual Sodium Carbonate ($mg\ L^{-1}$)

The RSC in t he groundwater varied between 0.14 to 4.95, 0.02 to 4.65, 0.02 to 6.25 and 0.17 to 2.78mg l^{-1} (Table 3) with a mean of 2.28[a], 1.67[a], 2.08[a] and 1.08[a]mg l^{-1} in agriculture, horticulture, barren and forest land use systems, respectively (Table 4). Out of 60 ground water samples collected 3 per cent samples were good for irrigation and classified as class RSC- 1, 38 per cent samples were classified as class RSC -2, 47 per cent samples were classified as class RSC- 3 and 7 per cent samples were classified as class RSC -4 in all land use systems (Table 7). A high range of RSC in groundwater indicates an increase in the adsorption of sodium in soil (Eaton, 1950).

Table 6

Ground water quality for sodicity hazard under different land use systems of Kolar district, Karnataka

Classes		Range ($mg\ l^{-1}$)	No of samples (%)				Total
			ALUS	HLUS	BLUS	FLUS	
S1	Ideal or excellent	< 10	15 (100)	15 (100)	15 (100)	15 (100)	60 (100)
S2	Good	10 - 18	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
S3	Doubtful	18 - 26	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
S4	Unsuitable	> 26	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Total No. of samples					60 (100)

Table 7

Ground water quality for alkalinity hazard/ index under different land use systems of Kolar district, Karnataka

Classes		Range ($mg\ l^{-1}$)	No of samples (%)				Total
			ALUS	HLUS	BLUS	FLUS	
Class I	Good	20	0	(0) 1	(7) 1	(7) 0	(0) 2 (3)
Class II	Permissible	20 - 40	7 (46.5)	5 (33)	6 (40)	5 (33)	23 (38)
Class III	Doubtful	40 - 60	7 (46.5)	7 (46.5)	8 (53)	6 (40)	28 (47)
Class IV	Unsuitable	60 - 80	1 (7)	2 (13.5)	0 (0)	4 (27)	7 (12)
		Total No. of samples					60 (100)

Table 8

Ground water quality for kelly's ratio under different land use systems of Kolar district, Karnataka

Classes		Range (mg l^{-1})	No of samples (%)				Total
			ALUS	HLUS	BLUS	FLUS	
KI-I	Not suitable	> 1	4 (27)	4 (27)	4 (27)	7 (47)	19 (32)
KI-II	Suitable	< 1	11 (73)	11 (73)	11 (73)	8 (53)	41 (68)
Total No. of samples							60 (100)

Kelly's Index (KI)/ Kelly's Ratio (KR)

Kelly's index is an indicator to assess irrigation water suitability, and it is free from the effect of K^+ parameter, which purely depends on Ca^{2+}, Mg^{2+} and Na^+. The average value of Kelly's index in groundwater was 0.82, 0.84, 0.73 and 1.06, respectively with a range of 0.39 to 1.76, 0.21 to 1.78, 0.21 to 1.33 and 0.28 to 1.58 in agriculture, horticulture, barren and forest land use systems, respectively (Table 3). In the entire study area, Kelly's index of about 32 (19 samples) per cent of samples were not suitable and 68 (41 samples) per cent of samples were suitable for irrigation (KI < 1) (Table 8). High KR in ground water samples was reduced by adding gypsum to the water samples to reduce the effect of Na^+ ion (Rawat *et al*., 2018).

Permeability Index (PI %)

Excess amount of Na^+, Ca^{2+}, Mg^{2+} and HCO_3^- in irrigation water affect soil permeability through widespread irrigation water use (Gautam *et al*., 2015). The average value of PI in groundwater recorded was 60.68^a, 63.55^a, 66.89^a and 69.80^a per cent in agriculture, horticulture, barren and forest land use systems, respectively (Table 4), which was categorized as good class (25 - 75 %) and ranged between41.96 to 88.95, 42.87 to 81.53, 45.57 to 80.98 and 48.58 to 88.11 per cent (Table 3) in agriculture, horticulture, barren and forest land use systems, respectively (good, PI: 25 -75 %). Permeability index range of 25 to 75 per cent indicates good irrigation efficiency of ground water (Table 9).

Sodium Per Cent

The sodium per cent of the groundwater samples varied from 27.46 to 62.96, 17.38 to 63.10, 16.80 to 56.44 and 21.18 to 68.09 per cent (Table 3) with an average of 42.28, 41.98, 39.59 and 46.75 per cent (Table 4) in agriculture, horticulture, barren and forest land use systems, respectively (Table 2). Out of 60 ground water samples collected 3 per cent samples were classified as good (Class I), 38 per cent samples were classified as permissible (class II), 47 per cent samples were classified as doubtful (Class III) and 12

Table 9

Ground water quality for permeability index under different land use systems of Kolar district, Karnataka

Classes		Range	No of samples (%)				Total
			ALUS	HLUS	BLUS	FLUS	
PI-I	Suitable	> 75	1 (7)	2 (13)	5 (33)	5 (33)	13 (22)
PI-II	Good	75 - 25	14 (93)	13 (87)	10 (67)	10 (67)	47 (78)
PI-III	Unsuitable	< 25	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total No. of samples							60 (100)

TABLE 10

Ground water quality for per cent sodium in Kolar gold fields, Karnataka

Classes		Range (mg l^{-1})	No of samples (%)				Total
			ALUS	HLUS	BLUS	FLUS	
Class I	Good	20	0 (0)	1 (7)	1 (7)	0 (0)	2 (3)
Class II	Permissible	20 - 40	7 (46.5)	5 (33)	6 (40)	5 (33)	23 (38)
Class III	Doubtful	40 - 60	7 (46.5)	7 (46.5)	8 (53)	6 (40)	28 (47)
Class IV	Unsuitable	60 - 80	1 (7)	2 (13.5)	0 (0)	4 (27)	7 (12)
Total No. of samples							60 (100)

per cent samples were classified as unsuitable (Class IV) for irrigation in all the land use systems (Table 10). Majority of water samples in the study area were not suitable for irrigation due to more leaching of Na from the rock and mining waste into the water (WHO, 2004).

A comprehensive assessment of groundwater quality in the Kolar gold mining area revealed significant spatial variation influenced by different land use systems. The pH of the water samples ranged from slightly acidic to neutral, indicating general suitability for drinking, irrigation and livestock purposes. However, prolonged mining activities and agricultural practices have the potential to disrupt this balance over time. The hydro chemical analysis indicated a dominance of cations in the order of Na^{+}> Ca^{+2}> Mg^{2+}> K^{+}, with sodium being the most prevalent, while bicarbonate (HCO_3^{-}) was identified as the dominant anion. Electrical conductivity (EC) measurements showed that most samples fell within the acceptable limits prescribed by the World Health Organization (WHO). Nonetheless, certain samples exceeded permissible thresholds, which may be attributed to anthropogenic inputs such as fertilizer application, mining effluents and sewage infiltration. Salinity hazard assessment classified a large proportion of groundwater samples under the high salinity category (C3), suggesting potential adverse effects on salt-sensitive crops. Although sodium adsorption ratio (SAR) values indicated excellent water quality across all samples (S1 category), parameters such as Residual Sodium Carbonate (RSC) and Kelly's Index highlighted localized concerns, indicating unsuitability for irrigation in specific areas. Overall, while the groundwater in the region is predominantly suitable for both irrigation and domestic use, the findings underscore the need for vigilant monitoring and sustainable water resource management. Anthropogenic pressures, particularly those associated with mining activities, intensive agriculture and wastewater discharge, pose a considerable risk to long-term groundwater quality.

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Farmer Strategies in Drought Affected Areas of North Eastern Dry Zone of Karnataka: A Socio-Economic Analysis

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ABSTRACT

Drought remains the most critical climatic risk to agriculture in the semi-arid regions of India particularly in the North Eastern Dry Zone (NEDZ) of Karnataka, where livelihoods are predominantly rainfed dependent. This study examined rainfall variability, socio-economic characteristics and coping strategies of 120 farmers from severely and moderately drought-affected villages in the NEDZ districts. The Standardized Precipitation Index (SPI) was employed to analyse annual rainfall data from 1991 to 2023. The results revealed that recurrent droughts with severe deficits occurring in 2003, 2012, 2015, 2018 and 2023 during which SPI values dropped below -1.5. Regarding socio-economic status, most of the respondents were middle-aged (average age 43 years), moderately educated and residing in joint families, with mean landholdings of 11.17 acres. Annual household income was generally low, with over 90 per cent of respondents earning less than Rs.3 lakh per annum, indicating high economic vulnerability. Coping mechanisms analysed using Garrett's ranking method, highlighted the adoption of drought-resistant crops as the most preferred strategy, followed by migration, crop insurance, early planting and crop diversification. Advanced irrigation, water conservation practices and government subsidies also played a supportive role. However, distress strategies such as borrowing and livestock sales were widely adopted during drought years, reflecting the fragility of rural livelihoods. The findings confirm that droughts are both frequent and severe in the NEDZ, significantly impacting farmers' socio-economic stability and compelling them to adopt short-term coping strategies rather than pursue long-term resilience. The study suggests that enhancing adaptive capacity through the promotion of climate-resilient cropping systems, targeted policy interventions and localized drought monitoring frameworks is essential to sustain agricultural productivity and secure rural livelihoods in Karnataka's drought-prone regions.

Keywords : Standardized Precipitation Index (SPI), Garette score, Deficit rainfall, Income

DROUGHT is a recurrent and complex climatic phenomenon that poses significant challenges to agricultural sustainability, rural livelihoods and overall economic development in India. As a predominantly agrarian economy, India is particularly susceptible to the impacts of drought, given that a substantial portion of its population depends directly or indirectly on agriculture for their livelihood. The agricultural sector accounts for nearly 50 per cent of the country's labour force and plays a critical role in ensuring food security, providing raw materials to various industries and supplying essential resources such as fuelwood, housing materials and medicinal plants (Lakshmi *et al.*, 2024).

Although droughts are experienced globally across various climatic zones, they are especially prevalent

and severe in tropical and sub-tropical regions. Among all natural hazards, drought is considered one of the most destructive, as it causes widespread economic losses, social distress and long-term environmental degradation. Unlike sudden-onset disasters, the onset of drought is gradual and often difficult to detect, as it lacks a clear demarcation from normal dry spells. It is generally recognized only after its adverse impacts become evident. Droughts, therefore, are insidious in nature, manifesting through prolonged periods of deficient precipitation that significantly impair water availability, crop production and ecosystem stability (Sengupta and Thangavel, 2023).

India's dependence on the southwest monsoon for the majority of its rainfall further exacerbates its vulnerability to drought. Variability in monsoon patterns frequently leads to droughts of differing intensity, particularly in arid, semi-arid and dry sub-humid agro-climatic regions (Das *et al*., 2024*)*. Understanding the frequency and intensity of droughts is essential not only for identifying recurrence trends but also for assessing the severity of water stress and its cascading effects on agricultural output, groundwater resources and national food security. This is especially pertinent in the Indian context, where approximately two-thirds of agricultural land is rainfed, making it highly sensitive to fluctuations in precipitation (Kumar *et al*., 2013). Consequently, evaluating drought patterns is fundamental to the formulation of effective drought risk management policies, promotion of climate-resilient farming practices and development of long-term adaptation strategies.

In this context, the present study examines the drought-prone North Eastern Dry Zone (NEDZ) of Karnataka. This zone has been selected for its varying degrees of drought severity, encompassing both severely and moderately affected areas. The study adopts an integrated approach that combines climatological assessment through the Standardized Precipitation Index (SPI) with socio-economic profiling of farmers. It further explores the adaptive and coping strategies employed by farming communities to manage drought-related risks. By synthesizing these dimensions, the study aims to provide a comprehensive understanding of livelihood vulnerability, the resilience of rural communities and the effectiveness of existing drought mitigation measures in the region. The insights derived are intended to inform targeted policy interventions and support the development of more sustainable and adaptive agricultural systems in drought-prone areas.

METHODOLOGY

Study Area and Sampling Framework

Study Area

The present study was conducted in the North Eastern Dry Zone (Zone-2) of Karnataka (Fig. 1), one of the most drought-prone agro-climatic zones of the state. This region frequently experiences delayed monsoons, erratic rainfall and recurrent droughts, which make agriculture highly vulnerable, especially for small and marginal farmers. The zone includes the districts like Kalaburagi, Yadgir, Raichur and Bidar where agriculture is the principal occupation. It is characterized by a semi-arid tropical climate, with an average annual rainfall ranging from 500 to 750 mm, most of which is received during the South-West Monsoon. The soils are predominantly black and red loamy, supporting rainfed cultivation of crops such as red gram, jowar, bajra, groundnut, sunflower and cotton.

Sampling Framework

The study adopted random sampling as the primary method for selecting the samples in the designated study area. Specifically, a simple random sampling technique was employed to ensure an unbiased and representative selection of sample respondents. A total of 120 respondents were chosen for the study, comprising 60 farmers from areas severely affected by drought and another 60 farmers from areas moderately affected by drought. This approach ensured balanced representation from both categories of drought-affected regions for comprehensive analysis.

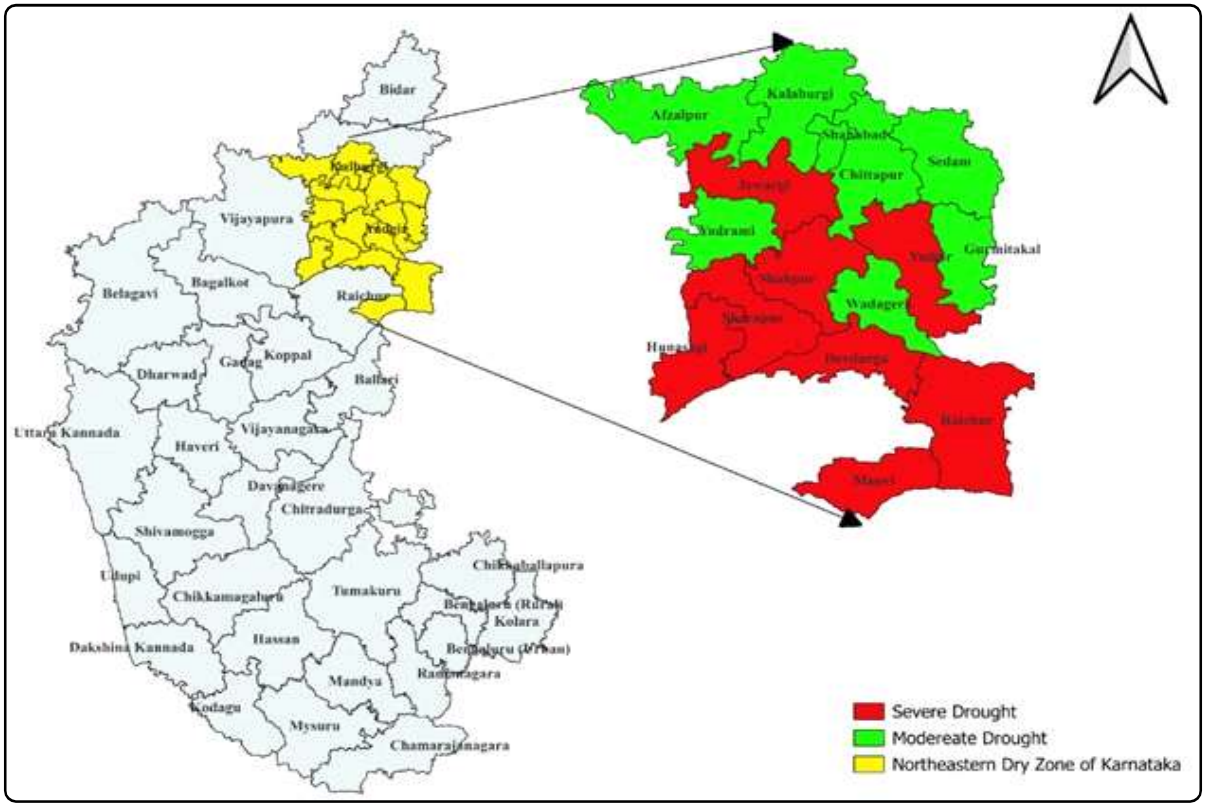

Fig. 1 : Map showing study area

TABLE 1

Distribution of respondents across the different drought regions

Drought Region	Taluk	Number of Samples (n)
Moderate Drought	Kalaburagi	15
	Yadgir	15
	Shahapur	15
	Devadurga	15
Severe Drought	Sedam	15
	Shorapur	15
	Raichur	15
	Manvi	15
	Total Sample Size	120

Analytical Tools and Techniques

Standardized Precipitation Index (SPI)

The SPI is a Z-score of precipitation, *i.e.,* it measures how much rainfall deviates from the historical average, expressed in standard deviation units (Bhushana, 2015).

$$SPI = \frac{(x - \bar{x})}{\sigma}$$

Where,

x = Rainfall for the period (e.g., current year rainfall)

$\bar{x}$ = Long-term mean rainfall

σ = Standard deviation of rainfall

Garrett's Ranking Technique

This technique was used to evaluate the coping mechanism adopted by the farmers to overcome drought. In this method, the farmers were asked to rank the given mechanism according to the magnitude. The orders of merit given by respondents were converted into ranks by using the following formula,

$$\text{Percentage Position} = \frac{100\,(R_{ij} - 0.5)}{N_j}$$

Where,

R_{ij} = Rank given for i^{th} measure by j^{th} individual

N_j = Number of measures ranked by j^{th} individual

The percentage position of each rank thus obtained was converted into scores by referring to the table given by Garrett. Then for each factor the scores of individual respondents were added together and divided by total number of respondents for whom the scores were added. These mean scores of all the factors were arranged in the order of their ranks and meaningful inferences were drawn (Madhu and Basavaraj, 2025).

Results and Discussion

The results on socio-economic profile of the respondents were presented in Table 2. The results of the analysis revealed that most of the farmers were in their middle-aged group, (70.83 %) of 35 to 50 years age group and the overall average age was 43 years. This indicated that most of them were experienced and possessed sufficient knowledge in agriculture. In contrast, only 17.50 per cent were younger (< 35) and 11.67 per cent were more than 50 years of age, suggesting a limited presence of both very young and older farmers. In terms of education, a significant portion (46.67%) completed only primary education, while 21.67 per cent had studied up to high school. The results are on par with the findings of (Ajayakumar and Gaddi, 2025) The proportion of respondents with college-level (15.83%) or graduation-level (3.33%) education was relatively low and 12.50 per cent were illiterate, highlighting the limited access to higher education among rural farmers. Regarding family structure, 61.67 per cent of the respondents lived in joint families with an average family size of 6, compared to 38.33 per cent living in nuclear families, which had a size of 3. Land holding analysis of respondents showed that only 5.83 per cent were marginal farmers (owning less than 2.5 acres), while 34.17 per cent had small holdings (2.5-5 acres), 25 per cent had semi-medium (5-10 acres) and 27.50 per cent had medium-sized farms (10-25 acres) and 7.50 per cent possessed, large holdings (> 25 acres). The average landholding across the sample was 11.17 acres, indicating a moderately distributed land base. The results on annual income of respondent family revealed that the income status of Rs.1.5 lakhs and Rs.1.5-3.0 lakhs together constituted more than 90 per cent.

Table 2

Socio economic status of farmers in the study area

(n=120)

Particulars	Number of respondents	Percentage	Average Value
Age			
<35	21	17.50	28.76
35-50	85	70.83	43.14
>50	14	11.67	57.71
Total	120	100.00	42.33
Education			
No formal education	15	12.50	-
Primary	56	46.67	-
Highschool	26	21.67	-
College	19	15.83	-
Graduation	4	3.33	-
Total	120	100.00	-
Family			
Joint	74	61.67	6.03
Nuclear	46	38.33	3.07
Total	120	100.00	4.89
Land holding (acres)			
Marginal (<2.5)	7	5.83	1.79
Small (2.5-5)	41	34.17	3.85
Semi medium (5-10)	30	25.00	7.95
Medium (10-25)	33	27.50	18.67
Large (>25)	9	7.50	32.67
Total	120	100.00	11.17
Annual Income (Rupees)			
<1.5 Lakhs	46	38.33	105264.39
1.5-3.0 Lakhs	65	54.17	223090.66
>3.0 Lakhs	9	7.50	347373.11
Total	120	100.00	187245.11

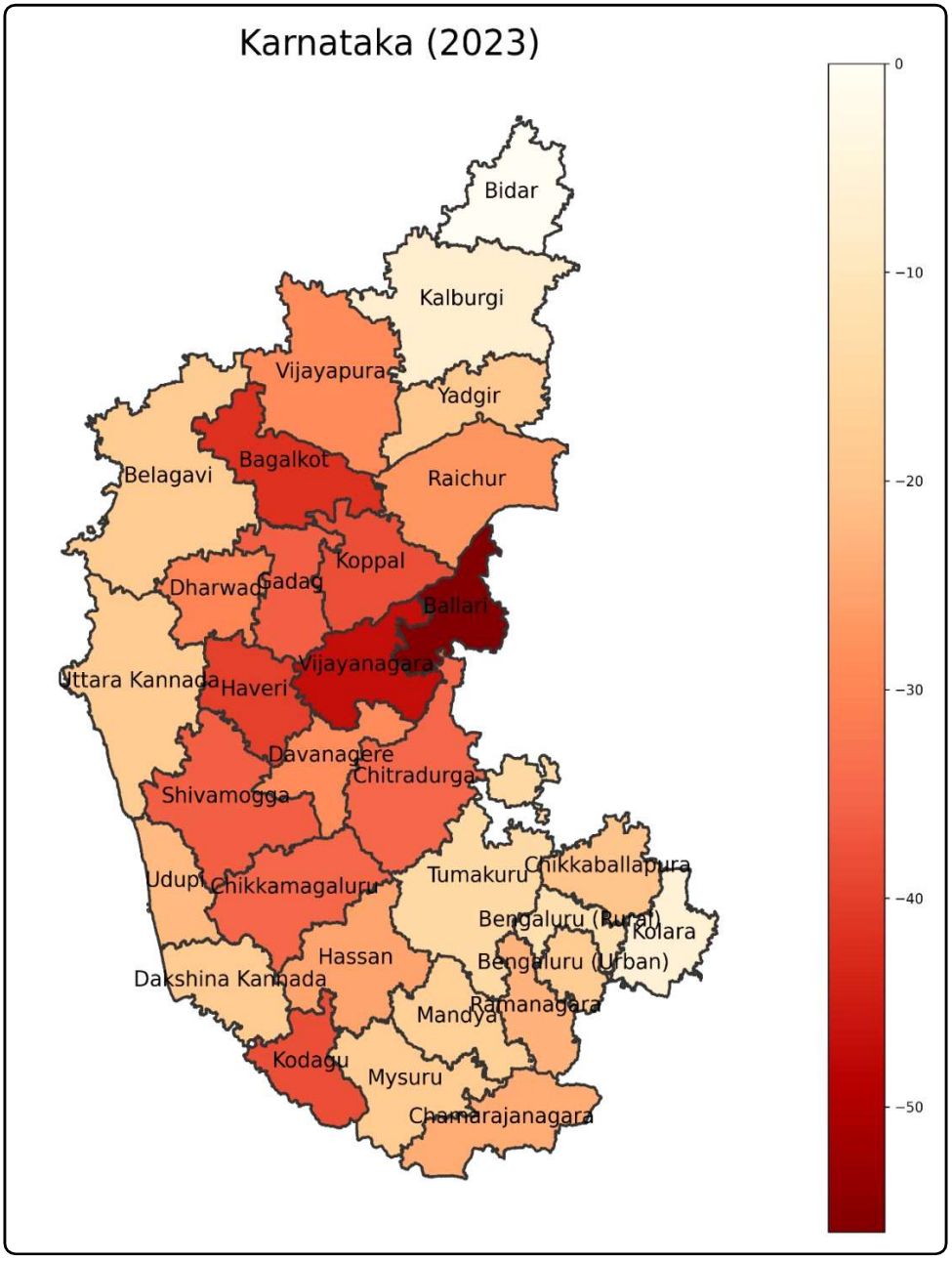

Fig. 2 : Deficit rainfall percentage in Karnataka (2023)

The average annual income of the respondents was Rs.1,87,245.11, which reflects the overall modest financial position of the farming households. Collectively, these findings suggest that the respondent group largely consists of middle-aged, moderately educated farmers living in joint families, with access to moderate land holdings but facing significant income constraints. These results are in line with that of (Lokesh, 2014).

The spatial distribution of rainfall deficit across the districts of Karnataka for the year 2023 is represented in Fig. 2. The colour intensity of each district corresponds to the deficit percentage indicated by the colour bar on the right: lighter shades of orange represent smaller deficits (closer to normal rainfall), while darker shades of red signify larger deficits, approaching -50 per cent or more.

This figure helps in identifying regions that experienced significant hydrological stress in 2023. The darker red areas, particularly evident in the North-Eastern Dry Zone of Karnataka which, include districts like Raichur, Kalaburagi and Yadgir indicate severe rainfall deficits. This suggests that these regions faced acute water scarcity, impacting agricultural yields, groundwater levels and potentially necessitating drought relief measures.

The analysis of rainfall and rainy months from 1981 to 2023 for Kalaburagi, Yadgir, Raichur and Karnataka were depicted in Table 3, the results showed that the Kalaburagi had the highest long-term average rainfall (757.44 mm), followed by Yadgir (689.38 mm) and Raichur (615.49 mm), while the state average was 662.92 mm. Although Karnataka received slightly less rainfall than Kalaburagi, it experienced more rainy months (average 11 rainy months per year) compared with the three districts, which suggested that rainfall at the state level was more spread out across months.

Annual rainfall in all three districts fluctuated strongly from year to year. The high rainfall, exceeding 1,000 mm per year was observed during 2020 and 2021, while drought years were observed during 1999, 2011 and 2018 which recorded below 500 mm annual rainfall. The average number of rainy months generally ranged from 7 to 9 per year but reached 11 to 12 months during very wet years such as 1981, 1997 and 2022. This pattern indicated both inter-annual variability and the occurrence of extreme events.

The Kalaburagi district received consistently more rainfall than Yadgir and Raichur, but it still had less rainy months than the Karnataka. Raichur emerged as the drought prone district, with both the lowest average rainfall and rainy months, making it more vulnerable to drought. The data also showed that a general decline in rainfall during the 1980s and early 1990s, followed by fluctuations and a rise in recent years. Notably, the period from 2020 to 2022 reflected anomalously high rainfall and longer rainy months across all areas.

TABLE 3

Trends and variability in rainfall and rainy months across NEDZ of Karnataka (1981-2023)

Year	Kalaburagi		Yadgir		Raichur		Karnataka	
	Total Rainfall	Rainy Months	Total Rainfall	Rainy Months	Total Rainfall	Rainy Months	Total Rainfall	Rainy Months
1981	1128.52	10.00	996.68	10.00	843.75	10.00	815.13	12.00
1982	812.11	8.00	764.65	8.00	675.00	8.00	709.78	11.00
1983	759.38	6.00	701.37	7.00	601.17	8.00	516.46	9.00
1984	648.63	7.00	611.72	6.00	532.62	7.00	608.16	12.00
1985	537.89	8.00	490.43	8.00	474.61	8.00	447.02	11.00
1986	643.36	9.00	601.17	11.00	516.80	11.00	621.10	12.00
1987	870.12	9.00	812.11	9.00	685.55	9.00	694.79	12.00
1988	791.02	9.00	706.64	9.00	632.81	9.00	653.71	11.00
1989	833.20	9.00	754.10	9.00	622.27	10.00	536.88	10.00
1990	1007.23	7.00	801.56	8.00	590.62	8.00	406.85	11.00
1991	648.63	6.00	590.62	7.00	558.98	6.00	643.30	9.00
1992	590.62	8.00	516.80	8.00	495.70	7.00	648.56	8.00
1993	838.48	9.00	743.55	9.00	648.63	9.00	704.01	11.00
1994	616.99	9.00	564.26	10.00	564.26	9.00	732.46	11.00
1995	1001.95	9.00	907.03	9.00	769.92	9.00	572.81	11.00

Continued....

TABLE 3 Continued....

Year	Kalaburagi		Yadgir		Raichur		Karnataka	
	Total Rainfall	Rainy Months	Total Rainfall	Rainy Months	Total Rainfall	Rainy Months	Total Rainfall	Rainy Months
1996	885.94	8.00	849.02	8.00	806.84	8.00	795.78	10.00
1997	569.53	11.00	564.26	11.00	543.16	11.00	551.22	11.00
1998	1033.59	6.00	933.40	8.00	827.93	8.00	632.04	10.00
1999	416.60	7.00	400.78	7.00	400.78	7.00	502.53	11.00
2000	743.55	7.00	701.37	7.00	664.45	8.00	647.87	12.00
2001	727.73	9.00	627.54	8.00	569.53	8.00	480.24	11.00
2002	632.81	7.00	616.99	7.00	543.16	8.00	423.71	11.00
2003	516.80	8.00	479.88	9.00	432.42	10.00	405.93	12.00
2004	706.64	10.00	606.45	9.00	537.89	9.00	565.84	11.00
2005	965.04	9.00	938.67	9.00	885.94	9.00	891.10	12.00
2006	785.74	9.00	717.19	9.00	643.36	9.00	736.03	10.00
2007	727.73	7.00	791.02	7.00	833.20	7.00	884.07	10.00
2008	849.02	10.00	791.02	10.00	727.73	10.00	743.14	11.00
2009	680.27	9.00	622.27	9.00	595.90	9.00	817.49	10.00
2010	975.59	9.00	849.02	9.00	690.82	9.00	858.45	11.00
2011	506.25	8.00	421.88	8.00	326.95	6.00	541.54	10.00
2012	558.98	8.00	490.43	8.00	411.33	8.00	528.54	10.00
2013	780.47	8.00	653.91	8.00	585.35	8.00	629.68	11.00
2014	733.01	11.00	690.82	11.00	659.18	10.00	741.57	11.00
2015	522.07	10.00	500.98	8.00	453.52	8.00	597.86	12.00
2016	817.38	8.00	743.55	8.00	638.09	6.00	541.65	11.00
2017	727.73	7.00	696.09	8.00	653.91	8.00	634.53	10.00
2018	421.88	8.00	406.05	8.00	305.86	8.00	583.16	12.00
2019	590.62	9.00	522.07	10.00	442.97	9.00	899.84	12.00
2020	1133.79	8.00	1102.15	9.00	1007.23	9.00	1104.88	12.00
2021	1108.39	9.00	971.22	9.00	767.83	10.00	944.65	12.00
2022	914.64	12.00	838.78	11.00	770.98	11.00	968.84	11.00
2023	810.08	10.00	553.98	10.00	527.26	10.00	542.49	11.00
Average	757.44	8.49	689.38	8.63	615.49	8.58	662.92	10.91

Source: NASA POWER

A clear spatial variation analysis of rainfall and rainy months (Thimmegowda *et al.,* 2025) across the three districts and Karnataka showed a rise in rainfall variability and extreme years over the last four decades. These patterns point to the importance of localised planning for agriculture, irrigation and drought management to cope with changing rainfall behaviour.

The detailed analysis of the annual rainfall patterns and the occurrence of drought years in the Kalaburagi district from 1981 to 2022 is depicted in Fig. 3.

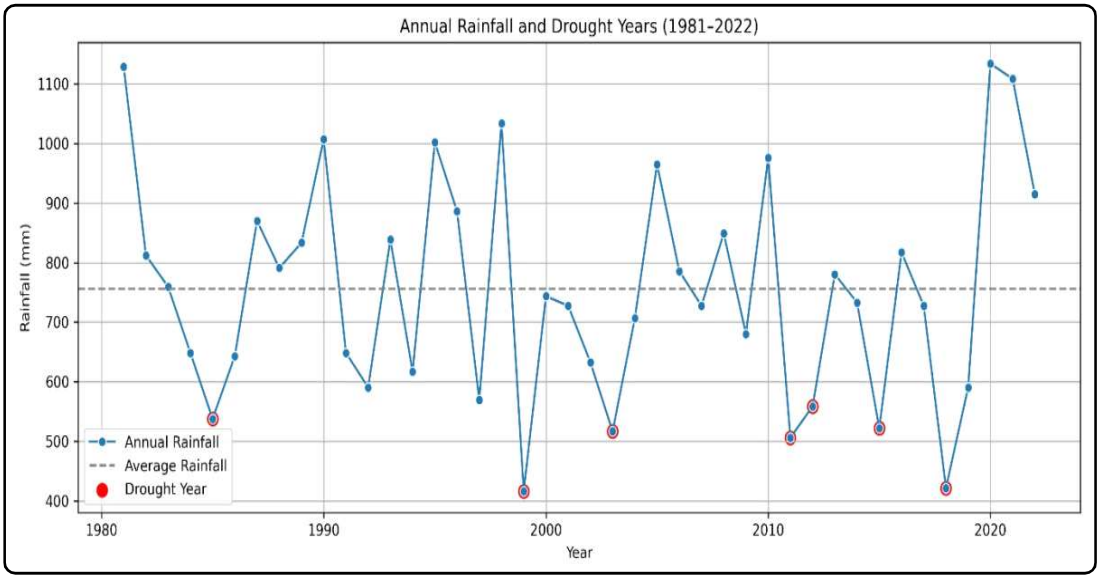

Fig. 3 : Rainfall distribution pattern in Kalaburagi

The results indicated that a long-term average rainfall of 760 mm per annum, which was the highest in NEDZ of Karnataka. The drought years identified were 1985, 1999, 2003, 2011, 2012, 2015 and 2018. The drought during 1999 and 2018 were very severe, with an annual rainfall of less than 450 mm. Kalaburagi also witnessed severe flood during 1981, 2020 and 2021 with an annual rainfall of greater than 1100 mm. The high variability of rainfall (from 400 mm to 1100 mm) over a period of 42 years indicated a significant challenge in water resource management in Kalaburagi.

The annual rainfall pattern analysis of Yadgir district from 1981 to 2022 was presented in Fig. 4, the results were similar to Kalaburagi district (1985, 1992, 1999, 2003, 2011, 2012, 2015 and 2018 were identified as drought years), with an average annual rainfall of 690 mm and the drought during 1999 and 2018 were identified as very severe with an annual rainfall of less than 400 mm, while the years 1981 and 2020 were observed as highest annual rainfall greater than 1000 mm. The frequent and recurring drought years contrasted with the extreme high rainfall years in

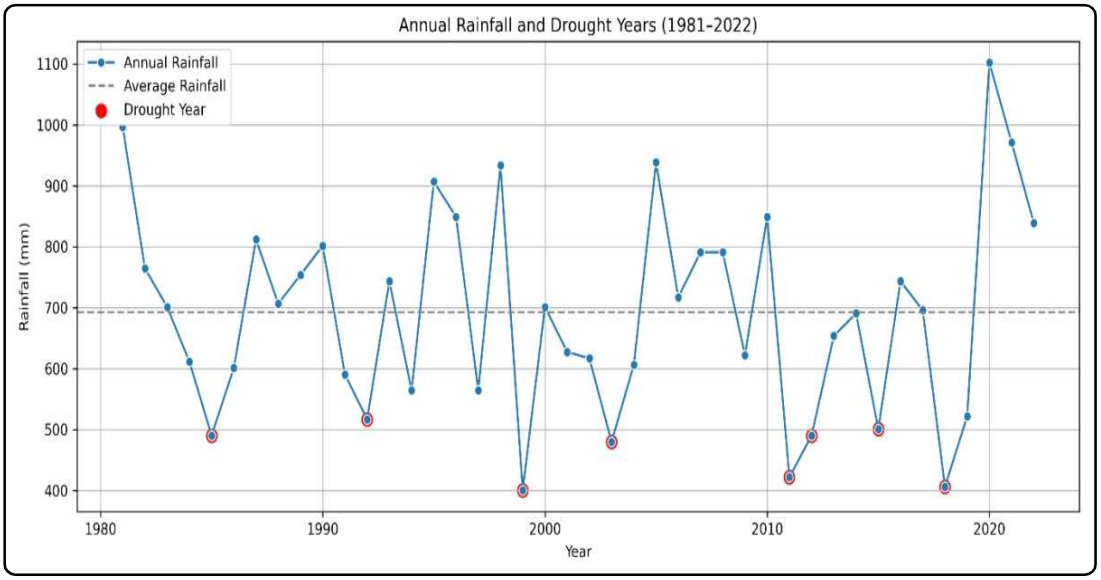

Fig. 4 : Rainfall distribution pattern in Yadgir

Yadgir district, pointed towards a high vulnerability to climate induced variability.

The rainfall distribution pattern for Raichur district from 1981 to 2022 (Fig. 5) showed a high variability with alternating dry and wet years over the period. The long-term average annual rainfall was 615 mm, which is the lowest in NEDZ Karnataka. Several drought years were 1999, 2011 and 2018 with annual rainfall below 400 mm, whereas high rainfall year was 2020 with an annual rainfall of greater than 770 mm. The total rainfall over the years varied widely, indicating fluctuating rainfall intensity. Overall, the Raichur district experienced a predominantly semi-arid climate with increasing inter-annual variability and occasional extreme rainfall variability in recent decades.

Fig. 6, showed the rainfall distribution pattern for Karnataka (1981-2022) and the results indicated that comparatively higher and more evenly distributed rainfall was seen in Karnataka than NEDZ. The long-term average annual rainfall was 663 mm. The years 2005, 2020 and 2022 recorded high rainfall exceeding 890 mm, whereas years identified as drought were 1885, 1990, 2001, 2002 and 2003 with an annual rainfall of less than 450mm. Despite some inter-annual variability, the state consistently maintained more rainy months, indicating a broader and more stable monsoon influence. Overall, the state of Karnataka experienced a relatively wetter climate pattern with increased rainfall intensity during the most recent decade compared with earlier periods.

The values of annual Standardized Precipitation Index (SPI) from 1991 to 2023 for Karnataka (state level) and the districts of Kalaburagi, Yadgir and Raichur are presented in Fig. 7. This is a strong and widely used tool to quantify drought intensity. The SPI is a standardized measure that identifies periods of precipitation surplus (positive values) or deficit (negative values), which helps detect drought affected years and droughts. In Kalaburagi, several years showed negative SPI values indicating drought *viz.,* 1993 (-1.69), 2000 (-1.17), 2003 (-1.17), 2009 (-1.23), 2012 (-1.14), 2018 (-1.67) and 2023 (-0.71) while, years such as 1996 (1.62), 2005 (1.22), 2010 (1.22) and 2020 (2.05) were wet years. Yadgir experienced notable droughts in 2003 (-1.31), 2011 (-1.76), 2015 (-1.88) and 2023 (-1.06), while extremely wet years like 2007 (1.80) and 2010 (2.85) were recorded as extreme wet years. In Raichur, severe drought years included 2003 (-1.58) 2012 (-1.94) and 2015 (-1.92), while 2005 (2.23) and 2020 (1.89) were very wet years. At the state level, Karnataka exhibited a mix of dry and wet years, with drought years such as 2003 (-1.54), 2012 (-1.40), 2018 (-1.77) and 2023 (-1.74) and wet periods in 2005 (1.85), 2010 (1.89) and 2020 (1.89) very wet years.

The SPI trends reinforce the recurring nature of drought in the study area and confirm historical

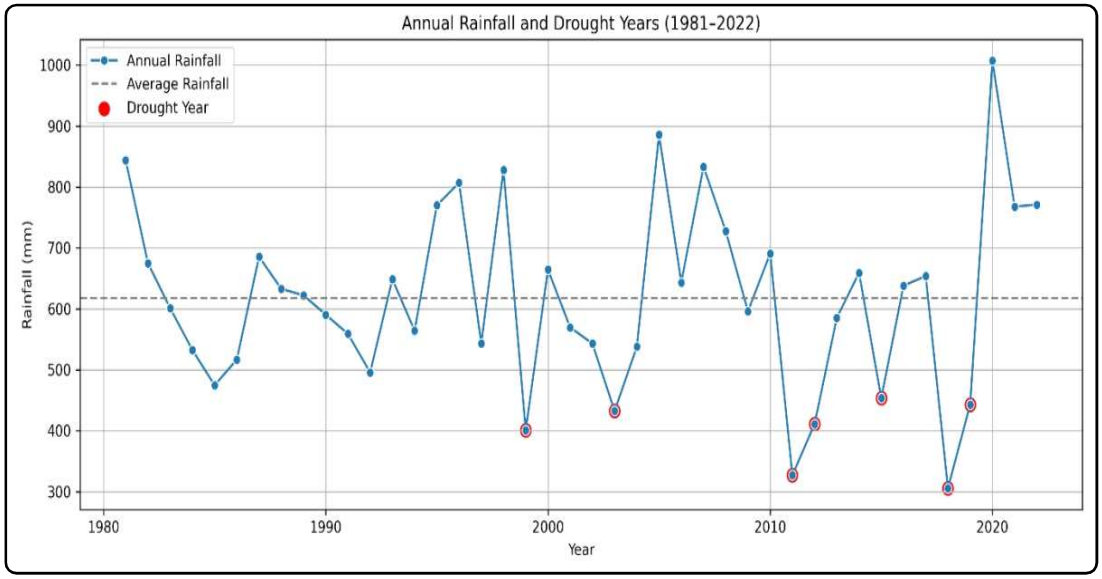

Fig. 5 : Rainfall distribution pattern in Raichur

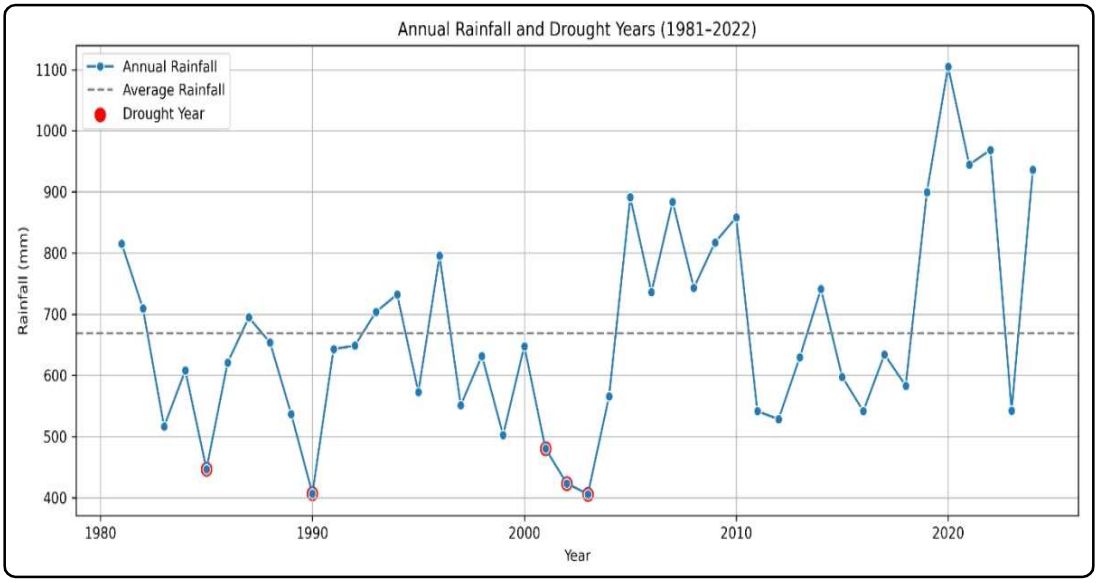

Fig. 6 : Rainfall distribution pattern in Karnataka

patterns of drought frequency and severity. The SPI values show frequent years with SPI below -1.5, especially 2002, 2015 and 2023, marking severe droughts. These values corroborate official drought declarations. Persistent negative SPI confirms the need for a localized drought-monitoring system.

The analysis of coping mechanisms adopted by farmers in response to drought was carried out using Garrett scores and results are presented in Table 3. The adoption of drought-resistant crops like jowar (CSV-37), bajra and redgram (TS-3R and Maruti) were emerged as the most preferred strategy (Garrett score: 53.42), reflecting a proactive shift towards climate-resilient farming. Migration ranked second (52.50), underscoring its role as a critical livelihood strategy during periods of agrarian distress. Other key responses included opting crop insurance (51.17), early planting (50.75) and crop diversification (50.08) like multiple cropping, changing cropping pattern and mixed farming, each aimed at mitigating climate-induced risks.

Similar coping behaviour was reported by (Lakshmi, 2016) in North Karnataka, where farmers widely adopted crop diversification, cultivation of drought-resistant varieties, protective irrigation and mixed cropping as primary strategies to withstand drought shocks. Technological adaptations such as advanced irrigation systems (49.83) and water conservation practices (48.67) also featured prominently, indicating a growing emphasis on resource management. Farmers further relied on institutional support through government subsidies and grants (48.67), while distress responses such as selling livestock/assets (48.50) and borrowing for livelihood needs (46.42) remained common.

The North Eastern Dry Zone of Karnataka is highly vulnerable to frequent and severe drought occurrences. The results of the Standardized Precipitation Index (SPI) over the period from 1991 to 2023 indicated persistent and recurrent deficits in rainfall across the region. Most farmers in this area are middle-aged, possess moderate landholdings and have moderate income levels. In response to drought conditions, farmers adopted various coping strategies, like cultivation of drought-resistant crop varieties, seasonal or permanent migration in search of alternative livelihoods and opting of crop insurance schemes. In addition to these adaptive measures, many farmers are compelled to resort to distress coping mechanisms such as taking loans and selling livestock assets to manage the immediate impacts of drought. To improve the resilience of farming communities and ensure agricultural sustainability, it is essential to strengthen climate-resilient agricultural practices, enhance policy support specifically targeted to drought-prone regions and establish localized drought monitoring and early

SPI in Karnataka, Yadgir, Raichur and Kalburgi (1991-2023)

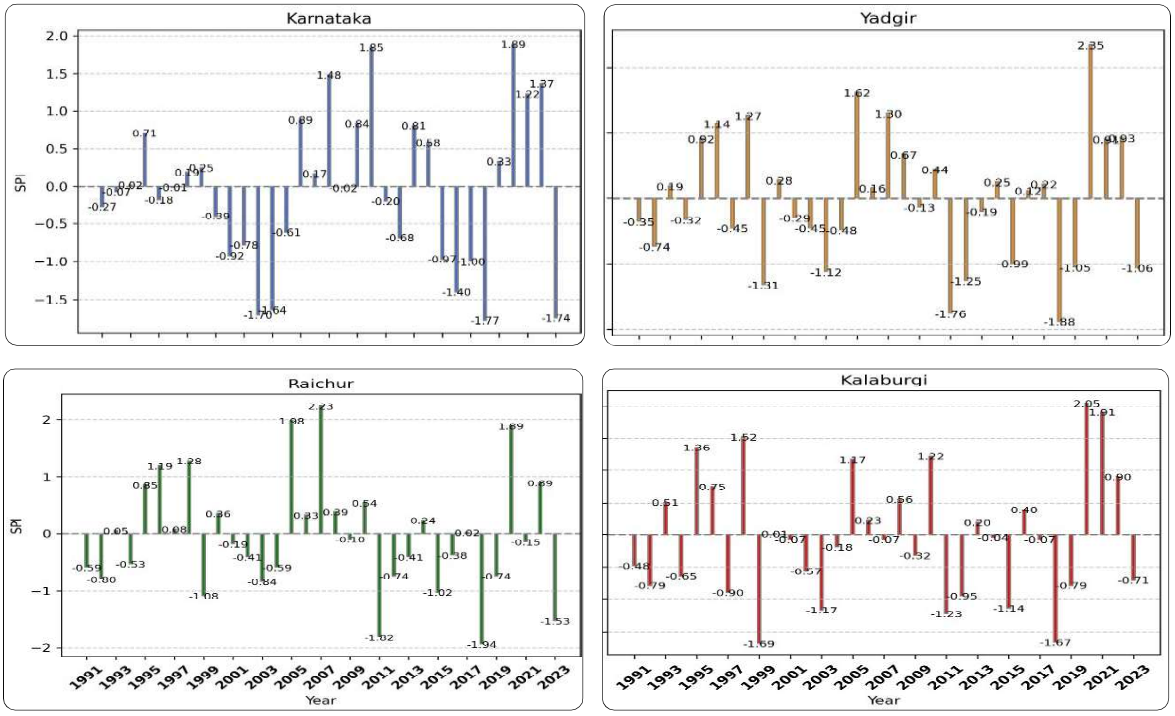

Fig. 7 : Standardized precipitation index (1991-2023)

warning systems. These interventions will play a critical role in safeguarding the livelihood security of farmers in the drought affected regions of the state.

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Attitudinal Assessment of Fish Farmers towards the Pradhan Mantri Matsya Sampada Yojana in Kalyana Karnataka

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Abstract

India's fisheries and aquaculture sector is experiencing rapid growth. However, small and marginal fish farmers continue to encounter significant constraints in input access, risk mitigation and market infrastructure. The Pradhan Mantri Matsya Sampada Yojana (PMMSY), launched to bridge these gaps through integrated interventions in production enhancement, post-harvest management, capacity building and social protection, represents a pivotal policy response. The study examined the attitude of fish farmers towards Pradhan Mantri Matsya Sampada Yojana (PMMSY) in the Kalyana Karnataka region of Karnataka. A total of 240 PMMSY beneficiaries (120 small and 120 big farmers) were selected from Raichur, Yadgir and Ballari districts using simple random sampling. Data were collected through a pre-tested structured interview schedule. Results revealed that 41.67 per cent of small farmers and 37.50 per cent of big farmers possessed favourable and highly favourable attitudes towards PMMSY scheme. Mann-Whitney U test indicated a significant difference in attitude between small and big farmers ($Z = 9.715$, $p < 0.001$). Satisfaction with departmental services, training programmes and input subsidies ranked highest, whereas e-consultancy services and cold storage facilities received the lowest ranks. The findings suggests that PMMSY scheme has positively influenced farmers' attitude, however strengthening extension delivery, simplifying procedures and improving post-harvest infrastructure are necessary to enhance scheme effectiveness.

Keywords : PMMSY, Attitude, Kalyana Karnataka, Impact, Aquaculture

India's fisheries and aquaculture sector has witnessed remarkable expansion, contributing 1.24 per cent to the national GDP and 7.96 per cent to agricultural GDP during 2022-23, with production reaching a record 182.12 lakh metric tonnes (Mt) comprising 119.99 lakh Mt from inland fisheries and 62.13 lakh Mt from marine sources. This growth trajectory, averaging 7.0 to 8.0 per cent annually over the past decade, positions India as the second-largest aquaculture producer globally, generating Rs.2.09 lakh crore in export earnings in the year 2022-23 and supporting livelihoods for over 28 million people, predominantly in rural coastal and inland regions (Anon., 2023). Aquaculture has transitioned from subsistence practices to commercial enterprises, fueled by rising domestic protein demand (per capita consumption: 19.70 kg/year), export markets (US$8.09 billion in FY23) and technological advancements like biofloc systems and genetically improved strains. Despite these achievements, small

and marginal fish farmers, who constitute 85.00 to 90.00 per cent of the sector's 4.5 million producers confront persistent structural constraints, including erratic water availability, escalating input costs, disease outbreaks, limited access to quality seed, inadequate technical knowledge and deficient market infrastructure. (Salam *et al.,* 2020).

These challenges exacerbate income volatility, with average smallholder earnings remaining below Rs.1.5 lakh/annum despite sector-wide growth. To address these gaps and achieve the National Fisheries Policy 2020 target of 22 million Mt production by 2024-25, the Government of India launched the Pradhan Mantri Matsya Sampada Yojana (PMMSY) in September 2020 with a Rs.20,050 crore outlay (2020-25). This flagship scheme targets holistic value chain enhancement through Rs.8,792 crores for infrastructure, post-harvest management, capacity building and social security. Digital innovations like e-consultancy portals and Matsya Sampada Kendra's further facilitate technology dissemination and advisory services. (Anonymous., 2023).

Although PMMSY has been widely implemented across India, empirical evidence on beneficiaries' attitudinal orientation towards the scheme remains limited, particularly in the Kalyana Karnataka region. Understanding farmers' attitudes is essential because favourable attitudes influence adoption behaviour, utilization of scheme components and long-term sustainability. Hence, the present study was undertaken to analyze the attitude of fish farmers towards PMMSY scheme and to compare the attitudes of small and big farmers. In addition, this study helped in identifying farm size differentials to inform targeted interventions for equitable sectoral growth.

METHODOLOGY

Study Area and Sampling Design

The study was conducted purposively in the Kalyana Karnataka region of Karnataka, which records the highest concentration of fish farmers in the state as per Directorate of Fisheries data. (Anonymous., 2023). Among the region, Raichur (349 registered farmers),Yadgir (133) and Ballari (122) districts exhibited the largest beneficiary bases under PMMSY scheme, hence this districts were selected for the study. From each district, two taluks were purposively selected and from each taluk 40 farmers (20 small and 20 big farmers) were selected through simple random sampling. Subsequently, 240 beneficiaries were selected out of which, 120 were small-scale farmers (pond size < 5 acres) and 120 were big farmers (pond size more than 5 acres). Primary data was collected through personal interviews using a pre-tested interview schedule.

Attitude Measurement and Categorization

A standardized attitude scale was developed specifically for the study and employed to assess the attitude of fish farmers towards the PMMSY scheme. Later, attitudinal responses were quantified using the Relative Importance Index (RII) to rank statement-wise attitude and derive composite attitude scores:

$$RII = \frac{\sum W}{AN} = \frac{5n_5 + 4n_4 + 3n_3 + 2n_2 + 1n_1}{5N}$$

Where, W = weighting as assigned on 5 point Likert's scale by each respondent

A = Highest weight (here it is 5)

N = Total number in the sample.

Descriptive Statistics : Mean, standard deviation, frequency and percentage were used to summarize the socio-demographic characteristics and attitude distributions of the respondents. Inferential analysis was carried out using the Mann-Whitney U test to compare attitude score distributions between small and Big farmers.

Mann Whitney U Test

The Mann-Whitney U test, is a non-parametric statistical test used to compare two independent groups to determine, if there is a significant difference between their distributions. The test compares the ranks of observations between the two groups. The formula to calculate the Mann-Whitney U statistic is:

$U_1 = n_1 \times n_2 + n_1 \times (n_1+1)/2 - R1$

$U_2 = n_1 \times n_2 + n_1 \times (n_1+1)/2 - R2$

Let n_1 and n_2 be the sample sizes of groups 1 and 2, respectively. R_1 and R_2 are the sum of ranks in groups 1 and 2. The U statistic is computed for both groups as U_1 and U_2.

Data Processing and Software

Data entry and cleaning were performed using MS Excel, with descriptive analyses (Mean, standard deviation, frequency and percentage) computed therein. Inferential statistics, including Mann-Whitney U test, normality assessment and graphical representations were executed using IBM SPSS Statistics v.26.0.

Results and Discussion

Overall Attitude of Beneficiaries Towards PMMSY Scheme

It is evident from Table 1 and Fig. 1, that nearly two-fifths (41.67%) of small farmers belonged to the favourable attitude category, followed by unfavourable (30.83%) and highly favourable (27.50%) categories respectively. This indicated that a majority of small farmers possessed positive orientation towards the scheme, reflecting their

Table 1

Overall attitude of beneficiaries towards PMMSY scheme

Attitude	Small farmers (n_1=120)		Big farmers (n_2=120)		Total (n=240)	
	f	%	*f*	%	*f*	%
Unfavourable (<71.12)	37	30.83	39	32.50	76	31.67
Favourable (71.12-78.57)	50	41.67	36	30.00	86	35.83
Highly Favourable (>78.57)	33	27.50	45	37.50	78	32.50

Mean = 74.85; S.D = 7.4

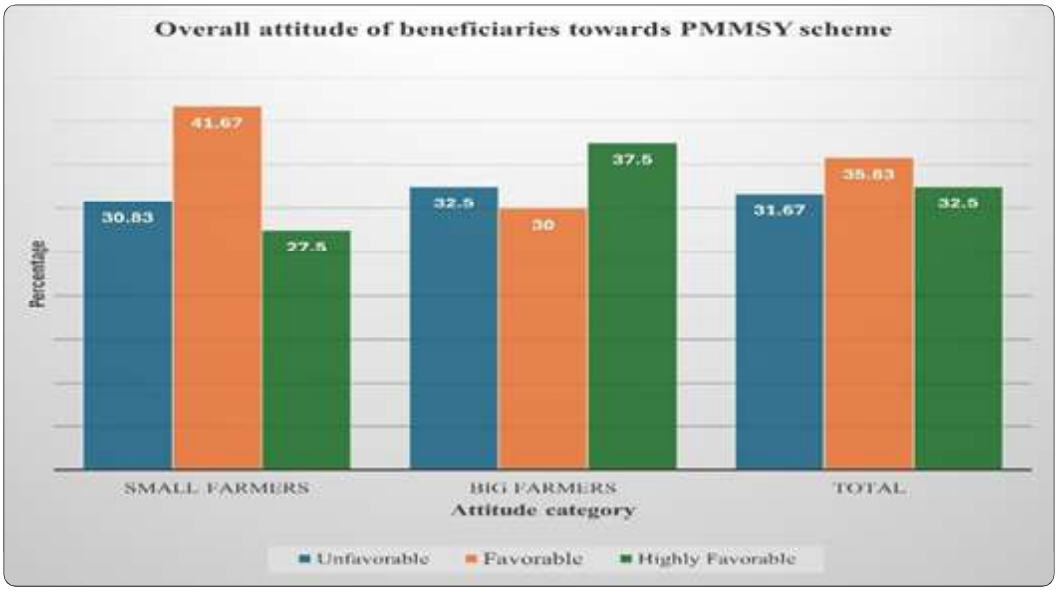

Fig. 1 : Bar chart representing overall attitude of beneficiaries towards PMMSY scheme

optimism regarding the developmental and livelihood enhancing opportunities provided under PMMSY scheme. The financial assistance offered through the scheme substantially reduced the overall cost of cultivation, which emerged as a major factor shaping favourable attitudes. Subsidies for pond construction and inputs such as fingerlings, feed and essential infrastructure effectively lowered the initial investment burden, enabling small farmers to engage in aquaculture with reduced financial risk.

In case of big farmers, more than one-third (37.50%) were categorized under the highly favourable attitude group, followed by 32.50 per cent with unfavourable attitude and 30.00 per cent with favourable attitude towards PMMSY scheme. The highly favourable attitude of big farmers can be attributed to their stronger resource base, better infrastructure and greater capacity to adopt improved aquaculture practices. Their larger pond holdings, assured water availability and prior exposure to departmental training programmes enabled them to utilize scheme components more efficiently, thereby reinforcing positive attitudes. Nevertheless, a small proportion of both small and big farmers expressed unfavourable attitudes, mainly due to delays in subsidy disbursement, procedural complexities, limited technical follow-up and weak market support.

At the overall level, more than one-third (35.83%) of the total farmers had a favourable attitude, followed by highly favourable (32.50%) and unfavourable (31.67%) attitudes towards PMMSY scheme. These findings suggested that farmers generally maintained a positive orientation towards the scheme, indicating that PMMSY scheme has succeeded in instilling confidence among beneficiaries regarding its potential to enhance livelihood security and fisheries development in the region. However, the presence of a considerable proportion of farmers with unfavourable attitudes highlights the need for improved awareness creation, transparent fund disbursement mechanisms and strengthened input supply systems to ensure equitable satisfaction and sustained participation across beneficiary categories. The results are in conformity with the findings of Suresh and Shivamurthy (2017), Kumar and Narayanagowda (2017) and Prabhulliger *et al.* (2017).

Comparative Analysis of Small and Big Farmer's Attitude Towards PMMSY Scheme

The comparative analysis of small and big farmers attitude towards PMMSY scheme presented in Table 2 and fig. 2 that, the Mann - Whitney U test revealed a highly significant difference in the attitude of small and big farmers towards the PMMSY scheme ($Z = 9.715$, $p < 0.001$). The marked variation in mean ranks, with big farmers recording

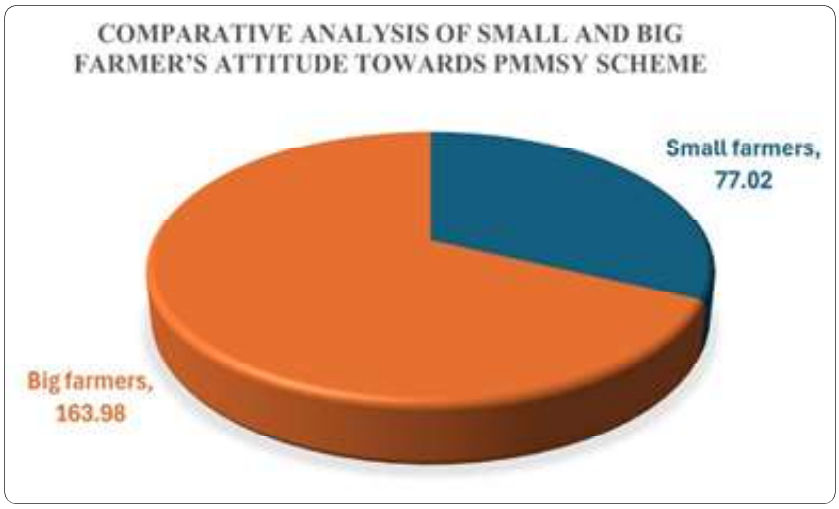

Fig. 2 : Comparative analysis of small and big farmer's attitude towards PMMSY scheme

TABLE 2

Comparative analysis of small and big farmer's attitude towards PMMSY scheme

Category	Sample size	Mean Rank	Sum of Ranks	Mann Whitney (Z value)	P value
Small farmers	120	77.02	9243	9.715 **	<0.001
Big farmers	120	163.98	19677		

**=Significant at 1% level

a substantially higher mean rank (163.98) than small farmers (77.02), indicated that big farmers possessed a considerably more favourable attitude towards the scheme. This difference is attributed to their relatively better resource base, higher capacity to utilize scheme components and greater exposure to institutional networks, which collectively enhance their confidence in and attitude towards the scheme benefits. In contrast, small farmers despite being beneficiaries faced constraints such as limited capital, lower risk-bearing ability and restricted access to information, which could moderate their attitude levels. Overall, the findings highlights that farm size plays an influential role in shaping the receptiveness and orientation of farmers towards the PMMSY scheme.

Statement Wise Attitude of PMMSY Beneficiaries Based on Relative Importance Index

Table 3 presents the relative importance of different PMMSY components as perceived by small and big farmers. Among small farmers, satisfaction with departmental services (Rank I), training programmes (Rank II) and capacity-building interventions (Rank III) received the highest priority, whereas e-consultancy services (Rank XX) and subsidized cold storage facilities (Rank XXI) were ranked lowest. Big farmers placed the highest importance to departmental services (Rank I), input subsidies (Rank II) and Processing of the fish products (Rank III), while e-consultancy services (Rank XX) and cold storage

TABLE 3

Statement wise attitude of PMMSY beneficiaries based on Relative Importance Index

Statements	Small farmers (n_1=120)		farmers (n_2=120)	
	RII	Rank	RII	Rank
Mass media, Exhibitions, Matsya mela, Tv, Radio and Newspaper coverage has created better awareness among beneficiaries about the scheme	0.617	XVII	0.655	XVI
Insurance companies, banks and fisheries department are not making adequate publicity regarding benefits of PMMSY	0.775	VI	0.642	XVII
Level of satisfaction with the services and benefits provided by the Department of Fisheries under PMMSY	0.952	I	0.860	I
Difficulty in understanding the procedure of becoming beneficiary of PMMSY	0.790	V	0.555	XIX
PMMSY makes me to feel confident through capacity building programmes to practice fish farming	0.830	III	0.792	VI
Matsya Sampada Kendra's provide timely advisory services to the fish farmers	0.558	XIX	0.660	XV

Continued....

TABLE 3 Continued....

Statements	Small farmers (n_1=120)		farmers (n_2=120)	
	RII	Rank	RII	Rank
e-consultancy services provided through the scheme regarding fisheries is timely and need based	0.510	XX	0.545	XX
Training programs given by PMMSY helps in acquiring knowledge and skills for optimum utilization of resources	0.848	II	0.778	VIII
PMMSY insurance reduces the market and production risk and uncertainty	0.657	XVI	0.800	IV
PMMSY help the farmers to sustain themselves from losses during unforeseen events through insurance for accidental disability, death and production losses due to climate vulnerabilities	0.742	XI	0.798	V
Subsides provided for purchasing inputs under this scheme are satisfying	0.747	X	0.842	II
Difficulty to claim insurance under PMMSY due to complicated procedures	0.727	XII	0.693	XIII
PMMSY encourages me to adopt innovative / modern technologies in fish farming for higher profits	0.692	XV	0.783	VII
PMMSY helps in practicing additional income generating activities like seed & feeding certification, e-marketing and e-trading	0.577	XVIII	0.700	XII
Subsidized fish cold storage facilities and processing units established under PMMSY benefited the farmers by avoiding delayed marketing losses	0.450	XXI	0.525	XXI
Scheme promoted me to practice diversification of the fish species for additional income	0.767	VII	0.757	IX
Processing of the fish products with the help of PMMSY can improve the economic security	0.810	IV	0.812	III
PMMSY provides employment opportunities to rural youth	0.758	IX	0.720	XI
PMMSY helps to provide quality fingerlings at affordable prices	0.763	VIII	0.735	X
Unwillingness to pay for PMMSY insurance due to low compensation	0.717	XIII	0.622	XVIII
Authorities need to reduce the formalities in claiming subsides	0.702	XIV	0.680	XIV

facilities (Rank XXI) received the least priority. The pattern indicates that farmers value PMMSY scheme's components that provide direct operational and financial benefits, whereas centralized infrastructure and digital services are yet to achieve effective field-level integration.

The results indicated that small farmers exhibit a stronger positive orientation towards PMMSY components that provide direct technical and capacity-building support. High importance accorded to departmental services, training programmes and confidence-building interventions.

It reflected the perceived reliability of institutional facilitation and the practical usefulness of knowledge transfer in improving farm-level management. Statements related to resource optimization and economic security also received relatively high priority, suggesting that small farmers recognize the tangible benefits of PMMSY-supported production and post-harvest interventions in enhancing operational efficiency and income stability. In contrast, low priority given to cold storage and processing facilities indicated limited functional access and weak integration of these infrastructure components at the village level.

Similarly, big farmers showed greater emphasis on financial and risk-mitigating components of PMMSY scheme, particularly input subsidies, insurance coverage and processing related economic security. This pattern suggested that large fish pond areas are better positioned to access scheme provisions and are more exposed to market and production risks, thereby valuing insurance and cost-reducing interventions more strongly. High importance assigned to institutional services further highlighted their dependence on formal support systems for enterprise expansion. However, low importance accorded to e-consultancy services and cold storage facilities points to persisting gaps in digital extension delivery and post-harvest infrastructure utilization even among larger farmers.

Statement Wise Attitude of Total Farmers Based on Relative Importance Index

It is evident from Table 4 that farmers expressed high satisfaction with PMMSY services (Rank I) and training programmes (Rank II), indicating a positive influence on knowledge enhancement and operational confidence among beneficiaries. Economic improvement through processing activities (Rank III), capacity-building support (Rank IV), input subsidies (Rank V), insurance support (Ranks VI and XI) and diversification (Rank VII) also received high priority, demonstrating the role of PMMSY in promoting income security and livelihood resilience. Statements related to awareness creation (Rank XVIII), insurance claim procedures (Rank XII) and institutional publicity (Rank XIII) received moderate rankings, suggesting persisting gaps in communication and administrative efficiency. In contrast, lower rankings for e-consultancy services (Rank XX), advisory services (Rank XIX) and subsidized cold storage facilities (Rank XXI) indicates that technological and infrastructural support systems require substantial strengthening to effectively meet farmers' practical needs. Overall, the pattern suggests that while PMMSY scheme has significantly benefited farmers, targeted improvements in administrative transparency and infrastructure development are essential for maximizing the scheme's impact.

TABLE 4

Statement wise attitude of total farmers based on Relative Importance Index

Statements	Total farmers (n=240)	
	RII	Rank
Mass media, Exhibitions, Matsya mela, Tv, Radio and Newspaper coverage has created better awareness among beneficiaries about the scheme	1.272	XVIII
Insurance companies, banks and fisheries department are not making adequate publicity regarding benefits of PMMSY	1.417	XIII
Level of satisfaction with the services and benefits provided by the Department of Fisheries under PMMSY	1.812	I
Difficulty in understanding the procedure of becoming beneficiary of PMMSY	1.345	XV
PMMSY makes me to feel confident through capacity building programmes to practice fish farming	1.620	IV

Continued....

TABLE 4 Continued....

Statements	Total farmers (n=240)	
	RII	Rank
Matsya Sampada Kendra's provide timely advisory services to the fish farmers	1.218	XIX
e-consultancy services provided through the scheme regarding fisheries is timely and need based	1.055	XX
Training programs given by PMMSY helps in acquiring knowledge and skills for optimum utilization of resources	1.627	II
PMMSY insurance reduces the market and production risk and uncertainty	1.457	XI
PMMSY help the farmers to sustain themselves from losses during unforeseen events through insurance for accidental disability, death and production losses due to climate vulnerabilities	1.540	VI
Subsides provided for purchasing inputs under this scheme are satisfying	1.588	V
Difficulty to claim insurance under PMMSY due to complicated procedures	1.420	XII
PMMSY encourages me to adopt innovative / modern technologies in fish farming for higher profits	1.475	X
PMMSY helps in practicing additional income generating activities like seed & feeding certification, e-marketing and e-trading	1.277	XVII
Subsidized fish cold storage facilities and processing units established under PMMSY benefited the farmers by avoiding delayed marketing losses	0.975	XXI
Scheme promoted me to practice diversification of the fish species for additional income	1.523	VII
Processing of the fish products with the help of PMMSY can improve the economic security	1.622	III
PMMSY provides employment opportunities to rural youth	1.478	IX
PMMSY helps to provide quality fingerlings at affordable prices	1.498	VIII
Unwillingness to pay for PMMSY insurance due to low compensation	1.338	XVI
Authorities need to reduce the formalities in claiming subsides	1.382	XIV

Statement Wise Attitude of Small and Big Farmers About PMMSY Scheme

The results presented in Table 5 indicated that the most favourable attitude among small farmers were directed towards the capacity enhancing components of PMMSY scheme, indicating their preference f or interventions that directly strengthen production and livelihood security. The highest agreement was recorded for fish species diversification (75.00%), reflecting farmers recognition of diversification as a strategy for income stabilization, followed by training programmes, with 73.33 per cent acknowledging their usefulness, suggesting the perceived effectiveness of skill-oriented interventions, processing-based economic security (70.83%), indicating confidence in value addition activities, input subsidies and the provision of quality fingerlings, each receiving 70.83 per cent agreement, highlighting the importance of affordable inputs in aquaculture operations. Capacity-building programmes (68.33%) and mass-media awareness efforts (69.17%) were also viewed positively, demonstrating the role of institutional support and information dissemination. Insurance in reducing market and production risks

TABLE 5

Statement wise attitude of small and big farmers about PMMSY Scheme

Statements	Small farmers (n_1=120)										Big farmers (n_2=120)									
	SA		A		UD		DA		SDA		SA		A		UD		DA		SDA	
	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%
Mass media, Exhibitions, Matsya mela, Tv, Radio and Newspaper coverage has created better awareness among beneficiaries about the scheme	47	39.17	36	30	10	8.33	15	12.5	12	10	45	37.50	35	29.17	12	10.00	20	16.67	08	6.67
Insurance companies, banks and fisheries department are not making adequate publicity regarding benefits of PMMSY	50	41.67	35	29.17	15	12.50	10	8.33	10	8.33	10	8.33	20	16.67	11	9.17	34	28.33	45	37.50
Level of satisfaction with the services and benefits provided by the Department of Fisheries under PMMSY	42	35.00	39	32.5	10	8.33	17	14.17	12	10	60	50.00	31	25.83	10	8.33	10	8.33	09	7.50
Difficulty in understanding the procedure of becoming beneficiary of PMMSY	45	37.50	33	27.5	11	9.17	18	15.00	13	10.83	60	50.00	25	20.83	15	12.50	12	10.00	08	6.67
PMMSY makes me to feel confident through capacity building programmes to practice fish farming	48	40.00	34	28.33	08	6.67	19	15.83	11	9.17	50	41.67	35	29.17	20	16.67	10	8.33	05	4.17
Matsya Sampada Kendra's provide timely advisory services to the fish farmers	43	35.83	30	25.00	11	9.17	26	21.67	10	8.33	32	26.67	28	23.33	18	15.00	24	20.00	18	15.00
e-consultancy services provided through the scheme regarding fisheries is timely and need based	34	28.33	39	32.5	11	9.17	18	15.00	18	15.00	43	35.83	32	26.67	19	15.83	11	9.17	15	12.50
Training programs given by PMMSY helps in acquiring knowledge and skills for optimum utilization of resources	49	40.83	39	32.5	13	10.83	11	9.17	08	6.67	36	30.00	46	38.33	15	12.5	12	10.00	11	9.17
PMMSY insurance reduces the market and production risk and uncertainty	54	45.00	24	20	10	8.33	19	15.83	13	10.83	46	38.33	36	30.00	15	12.50	10	8.33	13	10.83
PMMSY help the farmers to sustain themselves from losses during unforeseen events through insurance for accidental disability, death and production losses due to climate vulnerabilities	46	38.33	33	27.50	09	7.50	18	15.00	14	11.67	46	38.33	37	30.83	11	9.17	16	13.33	10	8.33
Subsides provided for purchasing inputs under this scheme are satisfying	51	42.50	34	28.33	10	8.33	14	11.67	15	12.50	44	36.67	36	30.00	15	12.50	14	11.67	11	9.17
Difficulty to claim insurance under PMMSY due to complicated procedures	53	44.17	20	16.67	15	12.50	14	11.67	18	15.00	51	42.50	33	27.50	09	7.50	15	12.50	12	10.00
PMMSY encourages me to adopt innovative / modern technologies in fish farming for higher profits	22	18.33	45	37.50	29	24.17	14	11.67	10	8.33	40	33.33	43	35.83	12	10.00	10	8.33	15	12.50

Continued....

TABLE 5 Continued....

Statements	Small farmers (n_1=120)										Big farmers (n_2=120)									
	SA		A		UD		DA		SDA		SA		A		UD		DA		SDA	
	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%	f	%
PMMSY helps in practicing additional income generating activities like seed & feeding certification, e-marketing and e-trading	15	12.50	17	14.17	43	35.83	29	24.17	16	13.33	40	33.33	35	29.17	08	6.67	18	15.00	19	15.83
Subsidized fish cold storage facilities and processing units established under PMMSY benefited the farmers by avoiding delayed marketing losses	45	37.50	35	29.16	14	11.67	11	9.17	15	12.50	48	40.00	30	25.00	10	8.33	17	14.17	15	12.50
Scheme promoted me to practice diversification of the fish species for additional income	51	42.50	39	32.5	09	7.50	11	9.17	10	8.33	52	43.33	35	29.17	07	5.83	15	12.50	11	9.17
Processing of the fish products with the help of PMMSY can improve the economic security	54	45.00	31	25.83	08	6.67	16	13.33	11	9.17	46	38.33	38	31.67	11	9.17	15	12.50	10	8.33
PMMSY provides employment opportunities to rural youth	48	40.00	31	25.83	12	10.00	19	15.83	10	8.33	38	31.67	35	29.17	20	16.67	15	12.50	12	10.00
PMMSY helps to provide quality fingerlings at affordable prices	51	42.50	34	28.33	14	11.67	09	7.50	12	10.00	40	33.33	36	30.00	19	15.83	15	12.50	10	8.33
Unwillingness to pay for PMMSY insurance due to low compensation	19	15.83	13	10.83	12	10.00	42	35.00	34	28.33	17	14.17	21	17.5	15	12.50	32	26.67	35	29.17
Authorities need to reduce the formalities in claiming subsides	18	15.00	16	13.33	14	11.67	31	25.83	41	34.17	20	16.67	16	13.33	17	14.17	36	30.00	31	25.83

SA- Strongly agree, A- Agree, UD- Undecided, D- Disagree, SD- Strongly disagree

received 65.00 per cent agreement, indicating moderate confidence in risk mitigation measures, while satisfaction with departmental services stood at 67.50 per cent, reflecting general approval of service delivery.

Moderate favorability was noted for e-consultancy services and advisory support from Matsya Sampada Kendras, each receiving 60.83 per cent, suggesting partial effectiveness of extension mechanisms, whereas modern technology adoption recorded 55.83 per cent agreement, indicating cautious acceptance of advanced practices. Additional income-generating activities attracted limited support (26.67%), possibly due to limited exposure and perceived feasibility. The least favourable attitudes were associated with negative statements such as inadequate publicity (70.84%), difficulty in understanding beneficiary procedures (65.00%), unwillingness to pay insurance premiums due to low compensation (26.66%) and procedural hurdles in subsidy claims (28.33%), reflecting notable administrative limitations.

In case of big farmers expressed the highest agreement for satisfaction with PMMSY scheme services, with 75.83 per cent endorsing this statement, indicating strong confidence in departmental support. This was followed by strong attitude regarding administrative procedures, as 70.83 per cent acknowledged difficulty in understanding beneficiary processes, reflecting perceived complexity in scheme implementation. Diversification of fish species received 72.50 per cent agreement, suggesting recognition of diversification as a pathway for income enhancement, while processing based economic security and insurance-related benefits each recorded 70.00 per cent agreement, indicating appreciation for value addition and risk mitigation measures. Adoption of modern technologies (69.16%) and the usefulness of training programmes (68.33%) were also well-supported, reflecting readiness to adopt improved practices. Moderate favourability was expressed for mass-media awareness (66.67%), availability of quality fingerlings (63.33%), additional income-generating activities (62.50%) and employment opportunities (60.84%), suggesting moderate satisfaction with information dissemination and livelihood support. Advisory services produced mixed responses, with 50.00 per cent agreement and 20.00 per cent disagreement, indicating variability in extension effectiveness. The least favourable attitudes were directed towards inadequate publicity (25.00%), unwillingness to pay insurance premiums due to low compensation (31.67%) and procedural complexities in subsidy claims (30.00%), indicating prominent administrative constraints despite overall favourable attitude of PMMSY scheme.

Statement Wise Attitude of Total Farmers About PMMSY Scheme

The results presented in Table 6 provide a comprehensive overview of the statement-wise attitude of total farmers towards the PMMSY scheme, reflecting their overall attitude towards various scheme components. Total farmers showed the most favourable attitude towards diversification of fish species (73.75%), satisfaction with PMMSY services (71.67%) and improvements in economic security through fish processing (70.42%) and insurance support (70.42%), reflecting strong appreciation of income-enhancing and risk-reducing interventions. Capacity-building programmes (69.58%) and training initiatives (70.84%) were also highly favoured, indicating recognition of the scheme's role in strengthening technical competence. Moderate favourability was observed for mass-media awareness (67.91%), availability of quality fingerlings (67.09%), subsidy benefits (67.09%), employment opportunities (63.33%) and adoption of modern technologies (62.50%), suggesting reasonable satisfaction with production and information support.

The least favourable attitudes were related to low insurance compensation (29.17%), procedural formalities in subsidy claims (29.16%), inadequate publicity (25.00%) and difficulties in insurance claims (30.00%), highlighting persistent administrative challenges. Overall, the findings suggest that the PMMSY scheme has generated a positive attitude among farmers, but improvements in administrative efficiency and extension support are required to enhance its overall effectiveness.

TABLE 6

Statement wise attitude of total farmers about PMMSY Scheme

Statements	Big farmers (n_2=120)									
	SA		A		UD		DA		SDA	
	f	%	f	%	f	%	f	%	f	%
Mass media, Exhibitions, Matsya mela, Tv, Radio and Newspaper coverage has created better awareness among beneficiaries about the scheme	92	38.33	71	29.58	22	9.17	35	14.58	20	8.33
Insurance companies, banks and fisheries department are not making adequate publicity regarding benefits of PMMSY	60	25.00	55	22.92	26	10.83	44	18.33	55	22.92
Level of satisfaction with the services and benefits provided by the Department of Fisheries under PMMSY	102	42.50	70	29.17	20	8.33	27	11.25	21	8.75
Difficulty in understanding the procedure of becoming beneficiary of PMMSY	105	43.75	58	24.17	26	10.83	30	12.50	21	8.75
PMMSY makes me to feel confident through capacity building programmes to practice fish farming	98	40.83	69	28.75	28	11.67	29	12.08	16	6.67
Matsya Sampada Kendra's provide timely advisory services to the fish farmers	75	31.25	58	24.17	29	12.08	50	20.83	28	11.67
e-consultancy services provided through the scheme regarding fisheries is timely and need based	77	32.08	71	29.58	30	12.50	29	12.08	33	13.75
Training programs given by PMMSY helps in acquiring knowledge and skills for optimum utilization of resources	85	35.42	85	35.42	28	11.67	23	9.58	19	7.92
PMMSY insurance reduces the market and production risk and uncertainty	100	41.67	60	25.00	25	10.42	29	12.08	26	10.83
PMMSY help the farmers to sustain themselves from losses during unforeseen events through insurance for accidental disability, death and production losses due to climate vulnerabilities	92	38.33	70	29.17	20	8.33	34	14.17	24	10.00
Subsides provided for purchasing inputs under this scheme are satisfying	91	37.92	70	29.17	25	10.42	28	11.67	26	10.83
Difficulty to claim insurance under PMMSY due to complicated procedures	104	43.33	53	22.08	24	10.00	29	12.08	30	12.50
PMMSY encourages me to adopt innovative / modern technologies in fish farming for higher profits	62	25.83	88	36.67	41	17.08	24	10.00	25	10.42
PMMSY helps in practicing additional income generating activities like seed & feeding certification, e-marketing and e-trading	55	22.92	52	21.67	51	21.25	47	19.58	35	14.58
Subsidized fish cold storage facilities and processing units established under PMMSY benefited the farmers by avoiding delayed marketing losses	93	38.75	65	27.08	24	10.00	28	11.67	30	12.50
Scheme promoted me to practice diversification of the fish species for additional income	103	42.92	74	30.83	16	6.67	26	10.83	21	8.75
Processing of the fish products with the help of PMMSY can improve the economic security	100	41.67	69	28.75	19	7.92	31	12.92	21	8.75
PMMSY provides employment opportunities to rural youth	86	35.83	66	27.50	32	13.33	34	14.17	22	9.17
PMMSY helps to provide quality fingerlings at affordable prices	91	37.92	70	29.17	33	13.75	24	10.00	22	9.17
Unwillingness to pay for PMMSY insurance due to low compensation	36	15.00	34	14.17	27	11.25	74	30.83	69	28.75
Authorities need to reduce the formalities in claiming subsides	38	15.83	32	13.33	31	12.92	67	27.92	72	30.00

SA- Strongly agree, A- Agree, UD- Undecided, D- Disagree, SD- Strongly disagree

TABLE 7

Statement-wise attitude scores and ranking patterns of small and big farmers

Statements	Small farmers (n_1=120)			Big farmers (n_2=120)		
	Total score	Mean score	Rank	Total score	Mean score	Rank
Mass media, Exhibitions, Matsya mela, Tv, Radio and Newspaper coverage has created better awareness among beneficiaries about the scheme	370	3.08	XVII	393	3.27	XVI
Insurance companies, banks and fisheries department are not making adequate publicity regarding benefits of PMMSY	465	3.88	VI	385	3.20	XVII
Level of satisfaction with the services and benefits provided by the Department of Fisheries under PMMSY	564	4.70	I	571	4.76	I
Difficulty in understanding the procedure of becoming beneficiary of PMMSY	474	3.95	V	333	2.77	XIX
PMMSY makes me to feel confident through capacity building programmes to practice fish farming	498	4.15	III	475	3.96	VI
Matsya Sampada Kendra's provide timely advisory services to the fish farmers	335	2.79	XIX	396	3.3	XV
e-consultancy services provided through the scheme regarding fisheries is timely and need based	306	2.55	XX	327	2.725	XX
Training programs given by PMMSY helps in acquiring knowledge and skills for optimum utilization of resources	509	4.24	II	467	3.89	VIII
PMMSY insurance reduces the market and production risk and uncertainty	394	3.28	XVI	480	4.00	IV
PMMSY help the farmers to sustain themselves from losses during unforeseen events through insurance for accidental disability, death and production losses due to climate vulnerabilities	445	3.71	XI	479	3.99	V
Subsides provided for purchasing inputs under this scheme are satisfying	448	3.73	X	505	4.21	II
Difficulty to claim insurance under PMMSY due to complicated procedures	436	3.63	XII	416	3.47	XIII
PMMSY encourages me to adopt innovative / modern technologies in fish farming for higher profits	415	3.46	XV	470	3.92	VII
PMMSY helps in practicing additional income generating activities like seed & feeding certification, e-marketing and e-trading	346	2.88	XVIII	420	3.50	XII
Subsidized fish cold storage facilities and processing units established under PMMSY benefited the farmers by avoiding delayed marketing losses	270	2.25	XXI	315	2.62	XXI

Table 7 Continued....

Statements	Small farmers (n_1=120)			Big farmers (n_2=120)		
	Total score	Mean score	Rank	Total score	Mean score	Rank
Scheme promoted me to practice diversification of the fish species for additional income	460	3.83	VII	454	3.78	IX
Processing of the fish products with the help of PMMSY can improve the economic security	486	4.05	IV	487	4.06	III
PMMSY provides employment opportunities to rural youth	455	3.79	IX	432	3.6	XI
PMMSY helps to provide quality fingerlings at affordable prices	458	3.82	VIII	441	3.675	X
Unwillingness to pay for PMMSY insurance due to low compensation	430	3.58	XIII	373	3.11	XVIII
Authorities need to reduce the formalities in claiming subsides	421	3.51	XIV	408	3.4	XIV

Statement-Wise Attitude Scores and Ranking of Small and Big Farmers

The results related to Attitude Scores and ranking of small and big farmers presented in Table 7 depicts that, small farmers expressed the most favourable attitude towards satisfaction with the services and benefits provided by the Department of Fisheries under PMMSY (Rank I), indicating strong acceptance of departmental support. This was followed by training programmes for acquiring knowledge, skills (Rank II) and confidence gained through capacity-building programmes (Rank III), suggesting that small farmers highly value skill-oriented and practical interventions. Processing of fish products for improving economic security (Rank IV) and difficulty in understanding the procedure of becoming a beneficiary (Rank V) were also ranked high, reflecting both perceived economic benefits and procedural challenges. Moderate importance was given to inadequate publicity regarding benefits of PMMSY (Rank VI), diversification of fish species (Rank VII), availability of quality fingerlings (Rank VIII) and employment opportunities to rural youth (Rank IX), showing recognition of livelihood and input-related support. The least favourable attitude was observed for subsidized fish cold storage facilities and processing units (Rank XXI) and e-consultancy services (Rank XX), indicating limited accessibility of these facilities.

Among big farmers, the highest ranked statement was satisfaction with the services and benefits provided by the Department of Fisheries under PMMSY (Rank I). This was followed by subsidies for purchasing inputs (Rank II) and processing of fish products improving economic security (Rank III), indicating greater emphasis on financial and value-addition support. Insurance reducing market and production risk (Rank IV) and insurance support during unforeseen events (Rank V) were also given high priority, highlighting the importance of risk management for large-scale operations. Moderate importance was assigned to adoption of modern technologies (Rank VII), training programmes (Rank VIII). The least favourable attitude was

TABLE 8

Statement-wise Attitude scores and ranking of Total farmers

Statements	Total farmers (n=240)		
	Total score	Mean score	Rank
Mass media, Exhibitions, Matsya mela, Tv, Radio and Newspaper coverage has created better awareness among beneficiaries about the scheme	763	3.18	XVIII
Insurance companies, banks and fisheries department are not making adequate publicity regarding benefits of PMMSY	850	3.54	XIII
Level of satisfaction with the services and benefits provided by the Department of Fisheries under PMMSY	1087	4.53	I
Difficulty in understanding the procedure of becoming beneficiary of PMMSY	807	3.36	XV
PMMSY makes me to feel confident through capacity building programmes to practice fish farming	972	4.05	IV
Matsya Sampada Kendra's provide timely advisory services to the fish farmers	731	3.05	XIX
e-consultancy services provided through the scheme regarding fisheries is timely and need based	633	2.64	XX
Training programs given by PMMSY helps in acquiring knowledge and skills for optimum utilization of resources	976	4.07	II
PMMSY insurance reduces the market and production risk and uncertainty	874	3.64	XI
PMMSY help the farmers to sustain themselves from losses during unforeseen events through insurance for accidental disability, death and production losses due to climate vulnerabilities	924	3.85	VI
Subsides provided for purchasing inputs under this scheme are satisfying	953	3.97	V
Difficulty to claim insurance under PMMSY due to complicated procedures	852	3.55	XII
PMMSY encourages me to adopt innovative / modern technologies in fish farming for higher profits	885	3.69	X
PMMSY helps in practicing additional income generating activities like seed & feeding certification, e-marketing and e-trading	766	3.19	XVII
Subsidized fish cold storage facilities and processing units established under PMMSY benefited the farmers by avoiding delayed marketing losses	585	2.44	XXI
Scheme promoted me to practice diversification of the fish species for additional income	914	3.81	VII
Processing of the fish products with the help of PMMSY can improve the economic security	973	4.05	III
PMMSY provides employment opportunities to rural youth	887	3.70	IX
PMMSY helps to provide quality fingerlings at affordable prices	899	3.75	VIII
Unwillingness to pay for PMMSY insurance due to low compensation	803	3.35	XVI
Authorities need to reduce the formalities in claiming subsides	829	3.45	XIV

recorded for subsidized fish cold storage facilities and processing units (Rank XXI) and e-consultancy services (Rank XX), similar to the pattern observed among small farmers. Overall, both small and big farmers exhibited a favourable attitude towards PMMSY, with small farmers prioritizing capacity building and training, while big farmers emphasized subsidies, insurance and processing related benefits.

Statement-Wise Attitude Scores and Ranking of Total Farmers

The data presented in Table 8 indicated that, among the total sample of beneficiaries the most favourable attitude was expressed towards level of satisfaction with the services and benefits provided by the Department of Fisheries under PMMSY scheme, which achieved the highest rank, followed by training programs given by PMMSY help in acquiring knowledge and skills for optimum utilization of resources (Rank II) and processing of fish products with the help of PMMSY scheme can improve the economic security (Rank III). Conversely, the statements that received the least importance from the total farmers were e-consultancy services provided through the scheme regarding fisheries is timely and need based (Rank XX) and fish cold storage facilities and processing units established under PMMSY benefiting the farmers by avoiding delayed marketing losses (Rank XXI).

The ranking pattern suggested that farmers placed greater value on PMMSY scheme's components that provide direct and immediate benefits to their farming operations. High ranking of satisfaction with departmental services reflects effective field-level support, guidance and accessibility of fisheries officials. The importance given to training programmes indicates farmers' reliance on capacity-building interventions to improve technical skills and resource utilization. Similarly, the high rank assigned to processing-related economic security highlights farmers' awareness of value addition as a pathway to income stabilization. On the other hand, the low ranking of e-consultancy services may be attributed to limited digital literacy, poor internet connectivity and preference for face-to-face interactions. The least importance assigned to cold storage and processing units suggests issues related to distance, accessibility, high operational costs and underutilization of infrastructure. Overall, the findings imply that strengthening field-based extension services, practical training and localized infrastructure would further enhance the effectiveness of PMMSY scheme.

The study establishes that the Pradhan Mantri Matsya Sampada Yojana (PMMSY) has successfully created a robust foundation for fisheries development in Kalyana Karnataka, evidenced by the fact that over two-thirds of beneficiaries maintain a positive attitudinal orientation. The scheme's primary success lies in its ability to reduce the financial risks of aquaculture through input subsidies and enhance human capital through effective training and capacity building programmes. However, the significant attitudinal disparity between small and big farmers ($Z = 9.715$, $p < 0.001$\$) highlights the critical need for more inclusive implementation strategies. While farmers express high satisfaction with core departmental services, the low rankings assigned to digital consultancy and post-harvest infrastructure highlight a disconnect between policy design and field-level utility. Consequently, the scheme's full potential is currently moderated by administrative complexities and digital divide. To transition towards an optimal impact, the programme must evolve from a subsidy-centric model to a streamlined, infrastructure heavy ecosystem that prioritizes the technological and procedural needs of small-scale producers.

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Genetic Variability and Correlation Studies for Yield and Yield Related Traits in Green Gram [*Vigna radiata* (L.) Wilckez]

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ABSTRACT

Genetic variability and association analysis are important tools in quantifying the degree of variance and knowing the individual contribution of the components traits on total variation. In the study, the set of 114 green gram genotypes were used, including two susceptible checks China mung and LM 1668. Field experiment was conducted in summer 2024 Mandya and analyzed for genetic variability, heritability, genetic advance and correlation analysis with respect to various important plant traits to identify high yielding genotypes for further stabilization and possible varietal release. The genotypes differed significantly for all the characters under study except days to fifty per cent flowering, number of primary branches and plant height as the population was derived from a single cross. The variation was recorded to be the highest for number of clusters per plant followed by number of primary branches per plant. The highest Genotypic coeffiecient of variance (GCV) and Phenotypic coeffiecient of variance (PCV) estimates were observed for all the traits except Days to fifty percent flowering, number of primary branches per plant, plant height and seed index. Seed yield per plant showed the highest positive association with pod yield, number of primary branches, plant height, number of clusters per plant and threshing percentage. However seed yield per plant showed significant negative correlation with days to fifty per cent flowering in the cross China mung × LM 1668. The present study suggests that traits like clusters per plant, pod yield and seed yield are influenced by additive gene action and are therefore heritable, indicating that selection for such traits will be rewarding for any breeding programmes.

Keywords : Genetic variability, Heritability, Yield traits of green gram

GREEN GRAM [*Vigna radiata* (L.) Wilczek] is ranked as the third most significant pulse crop in India. It is valued for its protein rich seeds (about 24/ g protein per 100/g), dietary fiber, antioxidants and micronutrients, as well as for its utility as a relay crop or green manure in rice-mung and maize-mung cropping systems, particularly in rainfed regions of India and Sub Saharan Africa. When combined with cereals, it constitutes a complete meal, offers a balanced profile of essential amino acids with high biological value. This crop is cultivated during both *kharif* and *rabi* seasons. The production and marketing of pulse crops hold substantial importance not only for the Indian economy but also for ensuring a balanced diet for the population. However, there has been a decline in the area and production of this crop due to various biotic, abiotic and management constraints. Biotic stresses, such as pests, diseases, and weeds, directly impact plant health and yield. Abiotic stresses, such as drought, salinity, extreme temperatures and nutrient deficiencies, can hinder plant growth and development. Additionally,

suboptimal management practices, such as inadequate irrigation, poor soil fertility management and lack of timely interventions, exacerbate these challenges. Yield is a critical factor in determining the success of crop improvement programs. To enhance yield potential adequate genetic variability within crop populations is essential (Ogunniyan and Olakojo, 2014). Estimates of genetic parameters provide insights into how the total variation of a character is influenced by different types of gene effects (Dagefa *et al.,* 2014). Quantitative traits are significantly affected by environmental factors, making it challenging to determine whether the observed variability is heritable or environmentally influenced. Therefore, high heritability is desirable for traits under selection. The results become more reliable and meaningful when coupled with genetic advance (Ogunniyan and Olakojo, 2014). For quantitative traits, indirect selection among different traits is more effective, which can be achieved through correlation studies. Correlation coefficient analysis provides the degree and relationship among important traits of interest and serves as a basis for selection (Hemavathy *et al*., 2015). However, it has limitations, as it only measures the mutual relation ship between two traits without implying the cause and effect of the relationship. To address this limitation, path coefficient analysis is employed as, it measures both the direct and indirect effects of association and estimates the relative importance of each causal factor. Thus, correlation and path analysis studies offer insights into the contribution of different plant traits to seed yield (Makeen *et al*., 2007). Consequently, the present investigation was undertaken to assess genetic variability, heritability, genetic advance and correlation analysis among important yield traits in recombinant inbred lines of green gram. The findings will aid in identifying high-yielding recombinant inbred lines (genotypes) for further stabilization and potential release as a variety.

Material and Methods

The experimental material comprised a set of 114 green gram genotypes, was sown in *kharif* 2024, carried out at K block, University of Agricultural Sciences, GKVK, Bengaluru. Geographically, the experimental site is located at 13° 00'N latitude and 77° 35'E longitude. The site is at an altitude of 899 m above mean sea level. The experimental material, as described above, was evaluated using an augmented block design, with two standard checks Chinamung and LM-1668 used for comparison. Each genotype was sown with a spacing of 30 cm × 10 cm. All genotypes derived from the cross between ABL-449 (DGGV-2 × IPM-2-14) and ABL-265 (a mutant of SM-17) were assessed for key yield and yield-related traits.

The recorded traits included days to fifty percent flowering, plant height (cm), number of primary branches per plant, number of clusters per plant, pod length (cm), and seed yield per plant (g) and seed index. Seeds from a randomly selected single plant within each line were harvested individually. Phenotypic observations were collected for the genotypes and the mean values of each trait were subjected to statistical analysis. The data was analyzed using R software, focusing on augmented design analysis, estimation of genetic variability parameters, and correlation studies for descriptive statistics.

Statistical Analysis

The data was subjected to statistical analysis. Mean, range, variance, coefficient of variance and standard deviation of the traits in greengram genotypes were estimated using 'Agicolae' package in R software (version 4.2.1.)

Variability Parameters

Broad sense heritability (h^2) was estimated using the following formula given by Allard (1960):

$b\ h^2 = \sigma^2 g / \sigma^2 p \times 100$

Where,

$\sigma^2 g$ = genotypic variance,

$\sigma^2 p$ = phenotypic variance

Phenotypic and genotypic coefficient of variation (PCV and GCV) for all the studied characters were

calculated by using the formula reported by Burton and De Vane (1953).

$PCV = (\sqrt{\sigma^2 p}/\mu) \times 100$ $GCV = (\sqrt{\sigma^2 g}/\mu) \times 100$

Where,

μ = Grand mean

Expected Genetic advance (GA) was figured using the following formula given by Allard (1960).

$GA = k \times h^2 \times \sqrt{\sigma^2} b$

Where,

k = selection differential (2.06) at 5% selection intensity

$b \sqrt{\sigma^2}$ = phenotypic standard deviation

The genetic advance as a percentage of the mean was estimated as:

$GAM = (GA\backslash\mu) \times 100$

Skewness and kurtosis statistics were estimated to understand the nature of distribution pattern of genotypes for yield its related traits, and to identify a superior segregant within the population under study. Skewness and kurtosis values were computed using the 'Agricolae' package in R software.

Correlation Coefficient Analysis

The correlation coefficients were estimated to understand the degree of relationship of characters with yield and component traits. The variance and covariance components were used to assess correlation coefficients between two characters at genotypic and phenotypic levels.

$$r_g(xy) = \frac{Cov_g(xy)}{\sqrt{\sigma_g^2(x) \cdot \sigma_g^2(y)}}$$

$$r_p(xy) = \frac{Cov_p(xy)}{\sqrt{\sigma_p^2(x) \cdot \sigma_p^2(y)}}$$

Where,

$r_g(xy)$ - genotypic correlation coefficient

$r_p(xy)$ - phenotypic correlation coefficient

$Cov_g(xy)$ - genotypic covariance of xy

$Cov_p(xy)$ - phenotypic covariance of xy

The calculated value of 'r' was compared with the table value at n-2 degrees of freedom at the 5 per cent level of significance where, n is the number of pairs of observation.

Results and Discussion

Analysis of Variance and Estimation of Variability in Greengram Genotypes

The analysis of variance revealed significant variation at 5 per cent probability level and highly significant variation at 1 per cent probability level. Most yield-attributing traits for green gram breeding lines exhibited significant variation. The progeny lines derived from the cross demonstrated high significance for traits such as the number of pods per plant (23.69), number of clusters per plant (12.64), pod length (12.26) and very high significance for seed yield per plant (12.48) and seed index (6.5) are presented in (Table 1). However, non-significant variation was observed for days to 50 per cent flowering, plant height and number of primary branches per plant (Table 1). The check also recorded highly significant variation for most traits. This pattern closely mirrors findings by earlier researchers. For instance, Priya and Babu (2024) reported similar findings revealing significant differences among the genotypes under study for all the traits except the number of branches per plant and days to maturity which aligns with the present results observed here for branching and flowering. Similar observations were also recorded by Gadakh *et al.* (2013), Prasanna *et al*. (2013), Ahmad *et al*. (2015), Aparna *et al.* (2015), Das and Barua (2015), Muralidhara *et al.* (2015), Aalok *et al.* (2017), Pavan *et al*.(2019), Pratap (2023) and Kumar *et al*. (2024).

Skewness and kurtosis are fundamental statistical measures used to evaluate the distribution characteristics of quantitative data. Skewness reflects the degree of asymmetry, while kurtosis describes the peakedness or flatness relative to a normal distribution. Most statistical software, including SPSS, reports *excess kurtosis*, which is obtained by subtracting 3 from the standard kurtosis value. A distribution is generally considered approximately

TABLE 1

ANOVA for yield and yield component traits of greengram genotypes

Source	Df	Mean. Sq							
		DFF	PH	PB	NP	NC	PL	SY	SI
Treatment (ignoring Blocks)	113	2.76 ns	46.1 ns	0.25 ns	11.55 *	4.26 *	3.49 *	3.7 **	0.23 **
Treatment: Check	1	0.29 ns	39.78 ns	0 ns	0.14 ns	3.21 Ns	0 ns	0 ns	0.29 **
Treatment: Test	111	2.74 ns	46.25 ns	0.26 ns	11.59 *	4.28 *	3.48 *	3.76 **	0.23 **
Treatment: Test vs. Check	1	7.68 ns	36.04 ns	0.03 ns	18.65 *	3.05 Ns	8.57 *	0.42 ns	0.16 **
Block (eliminating Treatments)	6	2.07 ns	129.09 *	0.4 ns	5.41 ns	1.13 Ns	2.35 ns	0.37 ns	0 ns
Residuals	6	1.45	19.72	0.24	3.11	1.08	0.83	0.5	0

ns P > 0.05; * P <= 0.05; ** P <= 0.01

DFF-Days to fifty percent flowering, PH- Plant height, PB-Primary branches, NP- number of pods per plant, NC- Number of clusters per plant, PL- Pod length, SY- Seed yield per plant, SI- Seed index

normal when skewness and excess kurtosis values range between -1 and + 1. In the present study, all yield-related traits exhibited near-normal distribution patterns, with the exception of seed yield and number of pods per plant. As illustrated in Fig. 1, these two traits showed negative skewness and leptokurtic behavior, suggesting the involvement of complementary gene action and the influence of a limited number of genes in their expression.

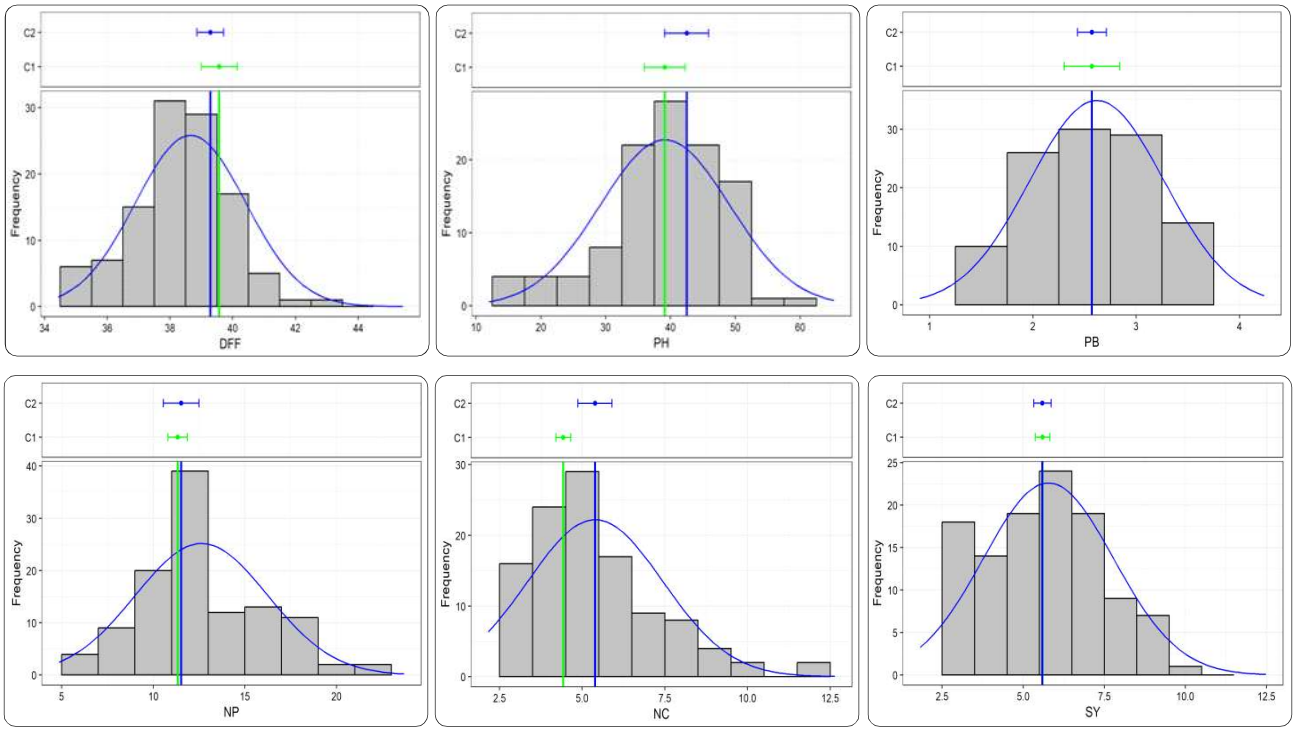

Fig. 1 : Frequency distribution of various quantitative traits in greengram genotypes

Table 2

Descriptive statistics of quantitative traits of Greengram genotypes

Trait	Count	Mean	Std.Error	Std.Dev	Min	Max	Skewness	Kurtosis
DFF	114	38.66	0.16	1.76	34.43	45.43	0.35 ns	4.34 *
PH	114	39.13	0.94	10.00	12.00	65.20	-0.54 *	3.86 ns
PB	114	2.62	0.06	0.65	0.90	4.24	0.02 ns	2.74 ns
NP	114	12.63	0.34	3.61	4.85	23.69	0.55 *	3.47 ns
NC	114	5.39	0.19	2.05	2.16	12.64	1.19 **	4.69 **
PL	114	5.67	0.18	1.95	1.34	12.26	0.91 **	4.27 *
SY	114	5.77	0.19	2.01	1.82	12.48	0.60 **	3.77 ns
SI	114	5.31	0.04	0.47	4.10	6.50	0.11 ns	2.62 ns

Estimates of PCV, GCV, Heritability and Genetic Advance for Yield Components in Greengram Genotypes

The genotypic and phenotypic coefficients of variation, heritability (broad sense) and genetic advance as a percentage of the mean for each character of the population are presented in Table 3. In the current study, significant variation was observed among the genotypes for eight quantitative characters recorded. The estimates of variability parameters were calculated according to the method suggested by Burton (1952). The significant differences indicate the presence of sufficient variability in the material under study.

The magnitude of the phenotypic coefficient of variation (PCV) exceeded that of the genotypic coefficient of variation (GCV) for plant height, number of primary branches, number of pods per plant, pod length, number of clusters per plant and seed yield per plant, suggesting that the apparent variation is influenced not only by genetic factors but also by environmental factors in the expression of these traits in mung bean. Higher estimates of GCV and PCV were observed for the number of pods per plant (23.04) and (26.95), the number of clusters per plant (33.18) and (38.35), pod length (28.71) and (32.88) and seed yield (31.27) and (33.58) respectively as shown in Fig. 2.

Table 3

Estimates of genetic parameters of various quantitative traits of greengram genotypes

Trait	Mean	PV	GV	EV	GCV	PCV	ECV	h2 (%)	GA	GAM	CV	MIN	MMAX x
DFF	38.66	2.74	1.28	1.45	2.93	4.28	3.12	46.92	1.6	4.14	3.11	34.43	45.43
PH	39.13	46.25	26.53	19.72	13.16	17.38	11.35	57.37	8.05	20.57	11.3	12	65.2
PB	2.62	0.26	0.02	0.24	4.68	19.31	18.74	5.87	0.06	2.34	18.77	0.9	4.24
NP	12.63	11.59	8.47	3.11	23.04	26.95	13.97	73.14	5.14	40.66	14.09	4.85	23.69
NC	5.39	4.28	3.2	1.08	33.18	38.35	19.24	74.82	3.19	59.2	19.41	2.16	12.64
PL	5.67	3.48	2.65	0.83	28.71	32.88	16.02	76.25	2.93	51.71	16.25	1.34	12.26
SY	5.77	3.76	3.26	0.5	31.27	33.58	12.23	86.74	3.47	60.09	12.27	1.82	12.48
SI	5.31	0.23	0.23	0	8.96	8.96	0	100	0.98	18.49	0.00	4.1	6.5

(PV - Phenotypic variance, GV - Genotypic variance, PCV - Phenotypic coefficient of variation, GCV - Genotypic Coefficient of variation, h2 - Broad sense heritability, GA - Genetic advance, GAM - Genetic advance as a percentage of the mean)

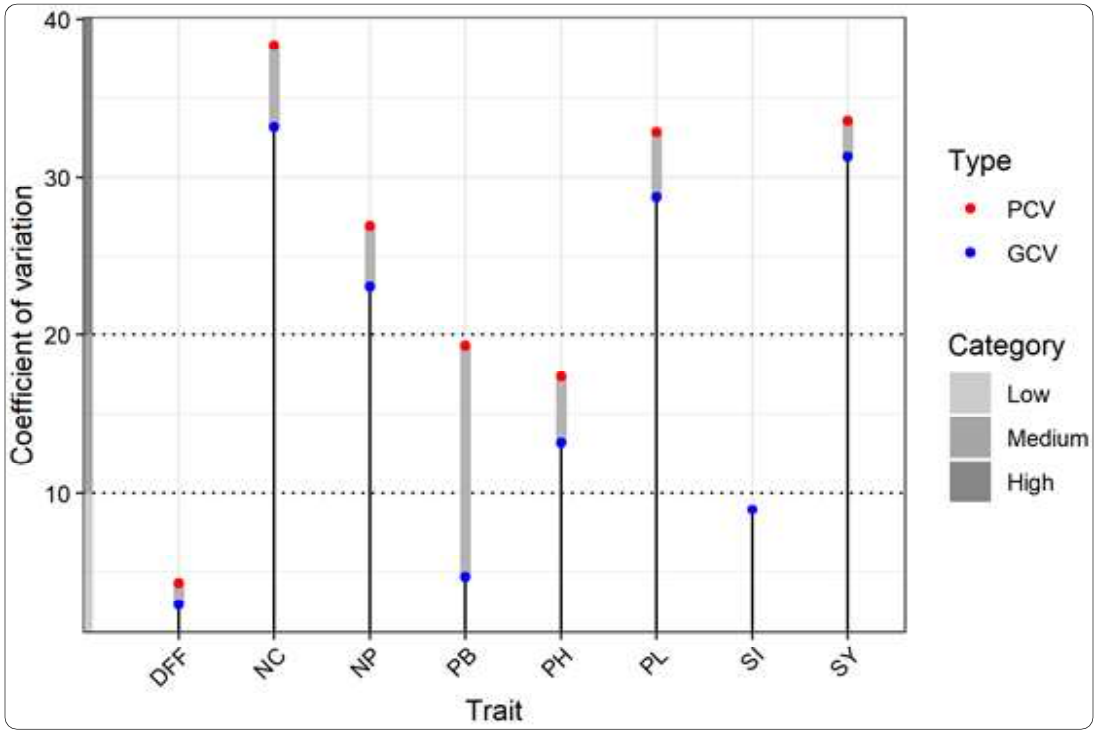

Fig. 2 : Coefficient of variation of various quantitative traits in greengram genotypes

Correlation studies among different yield attributing traits

In contrast, traits such as days to fifty per cent flowering and seed index recorded low GCV and PCV estimates (2.93 and 8.96) and (4.28 and 8.96), respectively. The magnitude of PCV and GCV merely indicates the extent of variation and not the heritable portion of the total variation present in the genetic material for different traits. In addition to PCV and GCV, the estimates of heritability and genetic advance for different traits provide more reliable information for their use as selection criteria in a breeding program. Similar to the present findings, Mariyappan *et al*. (2022) also reported high GCV and PCV for traits such as number of pods per plant, pod length and seed yield in mungbean. Moreover, high heritability coupled with high genetic advance for these traits emphasized the role of additive gene action and their potential utility in selection for crop improvement.

The broad-sense heritability was observed to be high for traits such as the number of pods per plant (73.14%), the number of clusters per plant (74.82%), pod length (76.25%) and seed yield (86.74%). In terms of genetic advance as a percentage of the mean, higher estimates were noted for all traits under investigation, with the exception of days to fifty percent flowering, the number of primary branches per plant, and seed index. The combination of heritability and genetic advance as a percentage of the mean provides a robust indication of the inheritance nature of a trait and the effectiveness of its selection in breeding. The traits of the number of pods per plant, number of clusters, pod length and seed yield exhibited high heritability coupled with high genetic advance, suggesting that these traits are predominantly governed by additive gene action and can be enhanced through simple phenotypic selection. Similar findings have been reported by Joseph *et al.* (2020), Dhunde *et al.* (2021), and Mariyappan *et al.* (2022).

Correlation Studies among different Yield Attributing Traits

Correlation coefficient analysis serves as a valuable tool in quantifying the degree of association between

key plant traits. It provides a foundational basis for formulating selection strategies, particularly when aiming to improve complex traits like yield. Understanding how various agronomic attributes interact with grain yield is essential for making informed breeding decisions, especially in crops like greengram where seed yield is influenced by multiple components (Khattak *et al.,* 2001). In the present investigation, correlation analysis was performed using trait-wise data collected from individual plants. The computed phenotypic correlation coefficients are presented in (Table 3). Results indicated that seed yield per plant exhibited a highly significant positive correlation with pods per plant, clusters per plant, plant height and primary branches per plant. These findings imply that selecting for these traits could effectively enhance seed yield in mung bean, as they contribute positively and directly to its expression.

A strong association of seed yield per plant with pods per plant, pod yield, primary branches and clusters per plant in mung bean has also been reported by many workers in diverse environments (Joseph *et al.,* 2020; Dhunde *et al.,* 2021 and Dhoot *et al.,* 2017). Therefore, the strategy of selection for component traits in mung bean should aim at the improvement of seed yield. However, efficiency of selection for component traits in increasing grain yield in mung bean depends not only on the strong association of component traits with grain yield, also the absence of any undesirable correlations among the components.

The study says that seed yield per plant has shown highly significant positive association in (Table 4) with pods per plant (0.29), clusters per plant (0.33), pod yield per plant (0.70), pod length (0.32), seed index (0.16) and clusters per plant (0.22), while it was negatively correlated with days to fifty percent flowering. A highly significant positive association was observed between pods per plant with number of primary branches per plant (0.26) and strong positive significant correlation with clusters per plant (0.54). Similar associations were reported by Prasanna *et al.* (2013) and Khajudparn and Tantasawat (2011), who observed a significant positive correlation of pods per plant with both number of primary branches and clusters per plant in mungbean. Their findings support the role of these yield components in enhancing overall productivity, thus validating their importance in selection strategies for mungbean

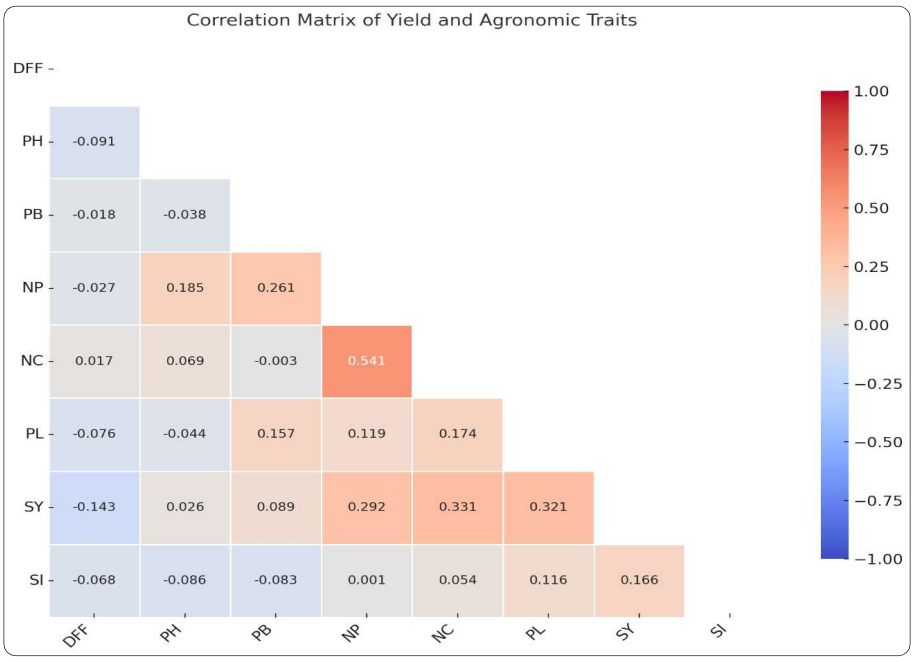

Fig. 3 : Heat map of Phenotypic correlation coefficients for seed yield and agronomic traits

TABLE 4

Phenotypic correlation study for yield and component traits in breeding lines of greengram

	DFF	PH	PB	NP	NC	PL	SY	SI
DFF	1	-0.091	-0.018	-0.027	0.017	-0.076	-0.143	-0.068
PH		1	-0.038	0.185 *	0.069	-0.044	0.026	-0.086
PB			1	0.261 **	-0.003	0.157	0.089	-0.083
NP				1	0.541 ***	0.119	0.292 **	0.001
NC					1	0.174 *	0.331 ***	0.054
PL						1	0.321 **	0.116 *
SY							1	0.166 *
SI								1

DFF-Days to fifty percent flowering, PH-Plant height, PB-Primary branches ,NP-number of pods per plant, NC-Number of clusters per plant, PL-Pod length, SY-Seed yield per plant, SI-Seed index

improvement. Similarly, there was a highly positive correlation for no. of pods per plant branches with no. of clusters per plant (0.89). whose results are in alignment with the Gadakh *et al.* (2013); Prasanna *et al*. (2013). Where seed index was found to be negatively significant correlated with plant height (-0.08), primary branches per plant (-0.083) and positively correlated with clusters (0.054) and pod length (0.11). Thus, the selection for plant types with more no. of seeds per plants, pods per plant, seeds per pod, pod yield per plant and clusters per plant is expected to ultimately increase seed yield in mung bean.

Therefore, this cross combination can be a good source for selection of desirable recombinants as large amount of genetic variability has been recorded and high heritability coupled with high genetic advance as a percentage of mean were observed for pod length, primary branches, pods per pod and clusters per plant suggesting genotypic variation for these traits due to high additive gene effects.

A significant positive association and high direct effect was noticed with pods per plant and clusters per plant on seed yield and revealed that the selection based on these traits would ultimately improve the pod yield. Thus, from the study it can be concluded that the traits like clusters per plant, seed yield and pod yield are influenced by additive gene action and are heritable which means selection for such traits will be rewarding for any breeding programmes.

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Effect of Halophilic Microbial Consortium Enriched with Expired Pharmaceutical Nutrient Supplements on the Growth and Yield of Rice in Sodic Soil

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Abstract

Sodic soils reduce rice productivity by causing ionic imbalances, osmotic stress and oxidative damage, which hinder nutrient uptake and physiological functions. Elevated sodium and pH levels further limit the availability of key micronutrients (Zn, Fe, Mn, Cu, B), compounding yield losses. Early-stage micronutrient supplementation, especially when integrated with halo-tolerant microorganisms, can enhance the nutrient solubility, uptake and plant resilience. To test this approach, a field experiment was conducted to evaluate the efficacy of halophilic microbial consortium consisting of *Bacillus subtilis*, *Halobacillu smassiliensis* and *Paraburkholderia fungorum* (S1) applied alone or enriched with either expired pharmaceutical nutrient supplements (EPNS) (S2: Bioron®, Bonetuff®, Huntred®, Lyvell®, Rymega-9G®) or conventional micronutrient salts (S3: $ZnSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, $MnSO_4$, $CuSO_4 \cdot 5H_2O$, $Na_2B_4O_7 \cdot 4H_2O$) equivalent to 1500:500:10:50:5 mM concentrations of Zn:Fe:Mn:Cu:B. The treatments were imposed via seed treatment (M1), soil application (M2), or a combination of both (M3), along with the recommended dose of fertilizers (RDF) in a 3 × 3 factorial randomized complete block design (RCBD) with nine treatment combinations plus a control and replicated three times. The combined seed and soil application of EPNS-enriched consortium (M3S2) produced the highest plant height (88.54 cm), number of tillers per m² (685), number of leaves per hill (88.32) and leaf area (870 cm² $hill^{-1}$). The yield parameters also improved significantly, the number of panicles per m² (280.8) and recording the lowest percentage of chaffiness (14.78%) resulting in the highest grain yield (48.56 q ha^{-1}) and straw yield (62.45 q ha^{-1}). These results demonstrate that integrating halophilic microbial consortium with EPNS could effectively address micronutrient deficiencies in sodic soils, improving plant growth and enhancing the rice yield potential.

Keywords : Halophilic microbial consortia, EPNS, Growth, Yield, Micronutreints

Sodicity is one of the most critical abiotic constraints limiting crop productivity and quality worldwide. In Karnataka, 1.48 million hectares are affected by salt-related problems of which sodicity accounts for over 200 hectares in the Cauvery Command Area of Mandya, a key rice-growing region (Jena & Natarajan, 2013). In this irrigated system, poor water management and continuous rice cultivation have intensified soil sodicity and alkalinity, resulting in nutrient imbalances, ion toxicity and reduced microbial activity. Rice yields in the state, averaging 3089 kg ha^{-1}, have remained stagnant due to progressive soil health deterioration (FAO, 2022; SRD, 2022 and Anonymous, 2023). High evaporation,

low organic matter and shallow saline sodic water tables accelerate sodium accumulation, impair photosynthesis and restrict crop growth (Hegde *et al*., 2015 and Rahman *et al*., 2017).

In sodic soils, excessive Na^+ and associated high pH displace essential cations such as Ca^{2+}, Mg^{2+} and K^+ severely disrupting plant physiological processes (Serrano *et al*., 1999 and Netondo *et al*., 2004). Sodium toxicity accelerates leaf senescence, reduces photosynthetic capacity, lowers water-use efficiency, and ultimately leading to significant yield losses (Munns, 2002; Shereen *et al*., 2005 and Gholipoor *et al*., 2002). It also impairs stomatal conductance, pigment stability and spikelet differentiation, particularly in inferior spikelets, thereby lowering the grain yield (Cattivelli *et al*., 2008 and Hayashi *et al.,* 2006). Although, rice plants deploy adaptive mechanisms such as osmolyte accumulation, ion homeostasis and reactive oxygen species (ROS), scavenging these are often inadequate under severe sodic stress (Golldack *et al.,* 2011 and Amirjani, 2011).

Conventional amelioration strategies such as the application of gypsum, organic matter and improved irrigation management are effective but often require community-level coordination for sustained impact. In contrast, the use of halophilic microbial consortium offers a more targeted approach for managing the microclimate of the rice rhizosphere in sodic soils (Tester & Davenport, 2003). These microbes produce Na^+-binding exopolysaccharides, phytohormones and stress-reducing enzymes (*e.g*., ACC deaminase), improving root zone conditions, nutrient uptake and overall plant resilience (Shah, 2016 and Varuna, 2021). When integrated with existing agronomic practices, halophiles can complement and enhance the efficacy of conventional approaches.

Micronutrients, though required in small quantities are critical for plant physiological and metabolic processes. Their deficiency, common in sodic soils, reduces the efficiency of applied N, P and K fertilizers, further constraining the growth and yield (Singh, 2008). High sodium levels and alkaline pH lower micronutrient solubility, while poor soil physical properties restrict root uptake, making integrated micronutrient management essential (Chand and Prasad, 2017).

Expired pharmaceutical nutrient supplements (EPNS), containing minerals such as Ca, Mg, S, Fe, Zn, Cu and Mn, represent an underutilized and sustainable alternative to conventional micronutrient salts. Repurposing these supplements not only supplies essential nutrients but also addresses the environmental concerns associated with pharmaceutical waste disposal. When combined with halophilic microbial consortia, EPNS can enhance nutrient availability through microbial chelation, sequestration and improved rhizosphere activity, while simultaneously mitigating the effects of sodic stress (Patel *et al*., 2021).

The present study was therefore undertaken to evaluate the efficacy of halophilic microbial consortium, either alone or in combination with EPNS or conventional sulfate salts, for improving rice growth and yield in sodic soils of the Cauvery Command Area in Karnataka.

Material and Methods

Experimental Site and Soil Characteristics

A field experiment was conducted during summer of 2024 at the College of Agriculture, V.C. Farm, Mandya, located in the Southern Dry Zone of Karnataka (12°342 N, 76°492 E; altitude 695 m above mean sea level). The experimental soil was sandy clay loam in texture, alkaline in reaction (pH 8.79), with an electrical conductivity of 0.34 dS m^{-1} and organic carbon content of 5.85 g kg^{-1}. The available nutrient status was low in nitrogen (236.43 kg ha^{-1}), medium in phosphorus (29.76 kg ha^{-1} P_2O_5), and medium in potassium (198.85 kg ha^{-1} K_2O), with an exchangeable sodium percentage (ESP) of 23.76. All DTPA-extractable micronutrients were above their respective critical limits Table 1.

Preparation of Halophilic Microbial Consortium Enriched with Micronutrients

Three most effective and compatible halophilic bacterial isolates, *Bacillus subtilis*, *Paraburkholderia*

TABLE 1

Initial soil physical and chemical properties of the experimental site

Particulars	Value
Physical properties	
Particle size distribution (per cent oven dry basis)	
Sand	59.73
Silt	12.09
Clay	28.18
Texture	Sandy Clay loam
Chemical properties	
Soil pH (1:2.5 soil water suspension)	8.79
EC (dS m^{-1}) (1:2.5 soil water suspension)	0.34
Cation exchange capacity [cmol (p^+) kg^{-1}]	16.16
Exchangeable sodium percentage	23.76
Organic carbon (g kg^{-1})	5.85
Available nitrogen ($kgha^{-1}$)	236.43
Available P_2O_5 (kg ha^{-1})	29.76
Available K_2O (kg ha^{-1})	198.85
Exchangeable calcium [cmol (p^+)kg^{-1}]	8.81
Exchangeable magnesium [cmol (p^+)kg^{-1}]	2.62
Exchangeable sodium [cmol (p^+) kg^{-1}]	3.84
Available Sulphur (mg kg^{-1})	14.19
DTPA-Fe (mg kg^{-1})	6.95
DTPA-Zn (mg kg^{-1})	1.01
DTPA-Mn (mg kg^{-1})	12.04
DTPA-Cu (mg kg^{-1})	2.79
Hotwater-soluble boron (mg kg^{-1})	0.87

fungorum and *Halobacillus massiliensis* were isolated from salt-affected soils of Mandya region and assayed for their compatibility with micronutrients using either expired pharmaceutical nutrient supplements (EPNS) or conventional micronutrient salts. Based on these assays, the most compatible concentrations were determined as 1500:500:10:50:5 mM Zn, Fe, Mn, Cu and B, respectively. The required quantities of EPNS (Bioron®, Bonetuff®, Huntred®, Lyvell® and Rymega-9G®) or conventional salts (zinc sulphate heptahydrate, iron sulphate heptahydrate, manganese sulphate, copper sulphate pentahydrate and disodium octaborate tetrahydrate) were accurately weighed, dissolved in sterile distilled water to prepare stock solutions and then diluted to the desired working concentrations. After inoculation of the isolates, the micronutrient-enriched cultures were incorporated into a talc-based formulation prepared using 0.17 mM NaCl to enhance viability. The prepared formulations were used for seed or soil application as per the experimental treatments.

Experimental Design and Treatments

The experiment was laid out in a 3 × 3 factorial randomized complete block design (RCBD) with nine treatment combinations plus a control, each replicated three times. The factors were:

-*Control treatment* consisted of RDF + $ZnSO_4 \cdot 7H_2O$ @ 40 kg ha^{-1} applied at transplanting.

-Factor 1: *Methods of application*

M1 : Seed treatment

M2 : Soil application

M3 : Seed treatment + soil application

-Factor 2: *Source of application*

S1 : Halophilic microbial consortium (HM-*Bacillus subtilis, Paraburkholderia fungorum, Halobacillus massiliensis*) alone

S2 : HM enriched with expired pharmaceutical nutrient supplements (HM+EPNS: Bioron®, Bonetuff®, Huntred®, Lyvell®, Rymega-9G® at 1500:500:10:50:5 mM Zn:Fe:Mn:Cu:B)

S3 : HM enriched with conventional micronutrient salts (HM+MM: $ZnSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, $MnSO_4$, $CuSO_4 \cdot 5H_2O$, $Na_2B_4O_7 \cdot 4H_2O$ at 1500:500:10:50:5 mM Zn:Fe:Mn:Cu:B)

For seed treatment, rice seeds @ 62.5 kg ha^{-1} were coated with 1 kg of halophilic microbial consortium, with or without enrichment as per treatment, using jaggery solution as a sticking agent prior to sowing in the nursery. For soil application, halophilic microbial consortia @ 10 kg ha^{-1} (with or without enrichment) were mixed thoroughly with farmyard manure and applied uniformly to the soil during transplanting.

Table 2

Interaction treatments for rice

Control	RDF
M1S1	RDF+ Seed treatment HM
M1S2	RDF+ Seed treatment EPNS+HM
M1S3	RDF+ Seed treatment MM+HM
M2S1	RDF+ Soil application HM
M2S2	RDF+ Soil application EPNS+HM
M2S3	RDF+ Soil application MM+HM
M3S1	RDF+ Seed treatment and Soil application HM
M3S2	RDF+ Seed treatment and Soil application EPNS+HM
M3S3	RDF+ Seed treatment and Soil application MM+HM

Crop and Nutrient Management

The rice variety 'MSN-99' was transplanted at a spacing of 20 × 10 cm using 30-day-old nursery seedlings (two seedlings per hill). The recommended dose of fertilizers for salt-affected soils (125:62.5:62.5 kg ha^{-1} $N:P_2O_5:K_2O$) was supplied using urea, di-ammonium phosphate (DAP) and muriate of potash (MOP) respectively, along with farmyard manure (10 t ha^{-1}). The calculated quantity of urea was applied in three split doses: 50 per cent of the recommended dose of nitrogen (RDN) as a basal application, 25 per cent at the tillering stage and 25% per cent at the panicle initiation stage. MOP was applied in two equal splits: half as basal and the remaining half at 60 days after transplanting (DAT). DAP was applied entirely as a basal dose. In addition, 50 per cent of the calculated gypsum requirement was incorporated uniformly during land preparation.

Observations Recorded

Morphological observations were recorded at critical growth stages 30, 60 and 90 days after transplanting (DAT) and at harvest. This included plant height (cm) and the number of tillers per hill at all observation stages, as well as leaf number and leaf area per hill at 30, 60 and 90 DAT. At harvest, yield data were collected from each net plot and grain and straw yields were estimated and expressed in quintals per hectare (q ha^{-1}). Yield-attributing parameters, namely the number of panicles per square meter and percentage chaffiness were also recorded at harvest. The collected data were subjected to statistical analysis following the methodology of Gomez and Gomez (1984).

Results and Discussion

Effect of Halophilic Microbial Consortium Eenriched with Expired Pharmaceutical Nutrient Supplements on Growth and Yield of Rice in Sodic Soil

Growth Parameters of Rice

Plant Height (cm)

The application of EPNS-enriched halophilic microbial (HM) consortium significantly influenced rice plant height at later growth stages (Table 3).

Table 3

Effect of expired pharmaceuticals and micronutrients enriched halophilic microbial consortium on plant height of rice at different growth stages

Treatments	Plant height (cm)			
	30DAT	60DAT	90DAT	At harvest
Methods of application				
M1	46.99	63.73	67.31	73.71
M2	48.42	70.05	75.01	79.81
M3	50.46	76.2	81.34	86.47
S.Em±	1.15	1.13	1.21	1.09
C.D @ 5 %	NS	3.28	3.51	3.17

Continued....

TABLE 3 Continued....

Treatments	Plant height (cm)			
	30DAT	60DAT	90DAT	At harvest
Source of nutrients				
S1	47.93	66.51	70.49	76.26
S2	49.08	72.06	76.79	82.13
S3	48.87	71.41	76.37	81.6
S.Em±	1.15	1.13	1.21	1.09
C.D @ 5 %	NS	3.28	3.51	3.17
T1 Control	46.12	53.34	56.77	63.89
S.Em±	1.89	1.89	2.03	1.83
C.D @ 5 %	NS	6.00	6.11	5.84
Interaction (M ×S)				
T_2 M1SI	46.76	59.77	63.14	70.11
T_3 M1S2	47.17	66.07	69.67	75.88
T_4 M1S3	47.05	65.34	69.11	75.14
T_5 M2S1	47.78	66.23	71.01	76.13
T_6 M2S2	48.89	72.15	77.12	81.97
T_7 M2S3	48.58	71.78	76.89	81.33
T_8 M3S1	49.25	72.54	77.33	82.55
T_9 M3S2	51.17	77.95	83.57	88.54
T_{10} M3S3	50.97	77.12	83.12	88.32
S.Em±	1.99	1.95	2.09	1.89
C.D @ 5 %	NS	5.68	6.09	5.49

While no differences were observed at 30 DAT, significant variation appeared at 60 DAT, 90 DAT and harvest.

Among the interactions, the highest, plant height was noticed in the treatment receiving seed treatment + soil application of HM+EPNS consortia (T_9), with 77.95 cm at 60 DAT, 83.57 cm at 90 DAT and 88.54 cm at harvest. These values were statistically on par with T_{10} (HM+MM with same method), which recorded 77.12, 83.12 and 88.32 cm, respectively. The lowest values were recorded in the control (T_1: 53.34, 56.77 and 63.89 cm).

Considering sources alone, HM+EPNS consortium (S2) recorded the highest plant height at 60, 90 DAT and at harvest (72.06, 76.79 and 82.13 cm), which was statistically on par with HM+MM consortium S3 and significantly higher than HM alone S1. Among the methods of application, the highest plant, height was recorded with seed treatment + soil application (M3: 76.20, 81.34 and 86.47 cm), followed by the soil application and seed treatment alone.

The accumulation of soluble salts such as sodium and chloride in sodic soils disrupts root architecture and limits water and nutrient uptake, especially potassium, calcium and manganese, thereby inhibiting plant growth (Karimi *et al.,* 2005). The high pH of these soils further aggravates nutrient deficiencies by reducing the availability of micronutrients like zinc (Zn), iron (Fe), manganese (Mn) and boron (B), impeding overall development. In this study, combined application of expired pharmaceutical nutrient

supplements (EPNS) and halophilic microbial consortium significantly improved plant height, indicating a synergistic role in alleviating salt stress. The halophilic microbes promote plant growth by producing phytohormones (IAA, gibberellins) that stimulate elongation, secrete extracellular polymeric substances (EPS) that bind sodium and enhance soil properties and generate ACC deaminase, which reduces ethylene levels that inhibit growth under stress (Shah, 2016 and Sarkar *et al.,* 2018). The micronutrients particularly zinc, boron and manganese are equally critical for plant height under sodic conditions. Zinc supports auxin synthesis and protein metabolism is essential for elongation (Ali *et al.,* 2011), boron maintains cell wall integrity and activates meristems (Hussain *et al*., 2012), while manganese aids enzymatic and metabolic functions for vegetative growth. These results align with earlier reports highlighting improved plant height through integrated nutrient management compared to conventional NPK alone (Meghana *et al*., 2019 and Zaman *et al*., 2020).

Number of Tillers per m^{-2}

The number of tillers per m^{-2} recorded no significant difference at 30 DAT, but significant variation was recorded at 60, 90 DAT and at harvest (Table 4). Among the sources, HM+EPNS (S2) produced the highest number of tillers (602, 612 and 615 m^{-2}). Regarding methods, seed treatment + soil application (M3) was most effective, resulting in the highest tiller numbers (634, 655 and 659 m^{-2}). Among the interaction treatments, the highest number of tillers was recorded in T_9 (HM+EPNS with seed treatment + soil application): 655 m^{-2} at 60 DAT, 677 m^{-2} at 90 DAT, and 685 m^{-2} at harvest. These were statistically on par with T_{10} (HM+MM with same method): 642, 665, and 672 m^{-2}, respectively. The control treatment (T_1) recorded the lowest number of tillers: 434, 441 and 450 m^{-2}.

The number of tillers per square meter improved significantly with the combined application of EPNS, micronutrient mixtures and halophilic microbial consortium. This enhancement is attributed to greater availability and mobility of key micronutrients,

Table 4

Effect of expired pharmaceuticals and micronutrients enriched halophilic microbial consortium on number of tillers of rice at different growth stages

Treatments	Tillers (Number m^{-2})			
	30DAT	60DAT	90DAT	At harvest
Methods of application				
M1	428	526	525	532
M2	445	583	590	589
M3	462	634	655	659
S.Em±	9.17	9.66	9.99	10.31
C.D @ 5 %	NS	28.07	29.03	29.97
Source of nutrients				
S1	438	551	559	558
S2	451	602	612	615
S3	447	591	600	607
S.Em±	9.17	9.66	9.99	10.31
C.D @ 5 %	NS	28.07	29.03	29.97

Continued....

Table 4 Continued....

Treatments	Tillers (Number m^{-2})			
	30DAT	60DAT	90DAT	At harvest
T1 Control	418.00	434.00	441.00	450.00
S.Em±	15.13	16.22	16.75	15.27
C.D @ 5 %	NS	51.87	53.57	46.91
Interaction (M ×S)				
T_2 M1SI	421	498	495	497
T_3 M1S2	432	549	547	551
T_4 M1S3	431	532	533	547
T_5 M2S1	434	551	559	555
T_6 M2S2	454	601	611	609
T_7 M2S3	448	598	601	602
T_8 M3S1	458	605	624	621
T_9 M3S2	466	655	677	685
T_{10} M3S3	463	642	665	672
S.Em±	15.88	16.72	17.29	17.86
C.D @ 5 %	NS	48.62	50.28	51.90

particularly zinc and boron, under microbe-assisted nutrient management. Zinc stimulates auxin-mediated bud outgrowth and cell division that is essential for tiller initiation, while boron supports meristematic activity and cell wall integrity, ensuring the emergence and stability of new tillers (Hussain *et al*., 2012 and Mohan *et al*., 2017). Additionally, the halophilic microbial consortium promote root development and nutrient uptake *via* IAA production and phosphate solubilization, indirectly fostering tiller proliferation (Tank & Saraf, 2010 and Bharti *et al*., 2016). These results are in agreement with the earlier findings that integrating NPK with micronutrients enhances tiller density more effectively than conventional fertilization (Zaman *et al*., 2020). In contrast, the poor performance under the control ($ZnSO_4$ alone) likely reflects zinc immobilization in sodic soils, highlighting the role of biological mediation for efficient nutrient delivery.

Leaf Numbers and Leaf Area per Hill

The effect of EPNS-enriched halophilic microbial consortium on leaf number and leaf area (cm^2 $hill^{-1}$) was evaluated across growth stages. No significant differences were noted at 30 DAT, but marked improvements appeared at 60 and 90 DAT as influenced by the method of application, sources of application and their interactions (Table 5).

Among the interactions, the seed treatment + soil application of HM+EPNS consortium (T_9) produced the highest leaf number (73.56 and 88.32 $hill^{-1}$) and leaf area (705 and 870 cm^2 $hill^{-1}$) at 60 and 90 DAT, respectively. These values were statistically on par with T_{10} (HM+MM consortia with the same application method), which recorded 72.21 and 87.31 leaves and 692 and 810 cm^2 leaf area. The lowest values were recorded in the control (T_1: 48.12 and 64.11 leaves; 450 and 579 cm^2). Considering the source of microbial enrichment alone, HM+EPNS consortium (S2) consistently outperformed others, recording 66.32 and 80.25 leaves $hill^{-1}$ and 637 and 777 cm^2 leaf area at 60 and 90 DAT, which was significantly higher than HM+MM and HM alone. With respect to the methods of application, the combined seed treatment + soil application (M3) showed the highest number of leaves (70.81 and 85.73

TABLE 5

Effect of expired pharmaceuticals and micronutrients enriched halophilic microbial consortium on leaves and leaf area of rice at different growth stages

Treatments	Leaves (Number hill^{-1})			Leaf area (cm^2 hill^{-1})		
	30DAT	60DAT	90DAT	30DAT	60DAT	90DAT
Methods of application						
M1	30.11	57.99	69.88	290	544	677
M2	31.72	63.45	76.93	303	614	727
M3	32.05	70.81	85.73	315	680	820
S.Em±	0.57	1.04	1.18	8.54	10.36	11.36
C.D @ 5 %	NS	3.01	3.43	NS	30.1	33.02
Source of nutrients						
S1	30.94	60.25	73.36	298	576	707
S2	31.62	66.32	80.25	307	637	777
S3	31.33	65.68	78.92	303	624	740
S.Em±	0.57	1.04	1.18	8.54	10.36	11.36
C.D @ 5 %	NS	3.01	3.43	NS	30.1	33.02
T_1 Control	29.33	48.12	64.11	282	450	579
S.Em±	0.94	1.74	0.62	14.09	17.4	19.3
C.D @ 5 %	NS	5.56	1.99	NS	55.67	60.88
Interaction (M×S)						
T_2 M1SI	29.35	53.97	66.17	287	510	641
T_3 M1S2	30.85	60.05	72.12	292	569	700
T_4 M1S3	30.14	59.94	71.34	290	553	690
T_5 M2S1	31.55	60.12	72.35	296	577	701
T_6 M2S2	31.88	65.34	80.32	310	638	760
T_7 M2S3	31.74	64.89	78.11	302	627	720
T_8 M3S1	31.92	66.67	81.57	311	642	780
T_9 M3S2	32.13	73.56	88.32	318	705	870
T_{10} M3S3	32.1	72.21	87.31	316	692	810
S.Em±	0.98	1.79	2.04	14.79	17.94	19.68
C.D @ 5 %	NS	5.21	5.94	NS	52.14	57.2

hill^{-1}) and leaf area (680 and 820 cm² hill^{-1}), followed by the soil application alone and seed treatment.

The number of leaves and area per hill that are the key indicators of vegetative vigor and photosynthetic potential, were significantly enhanced under treatments with EPNS and halophilic microbial inoculants compared to the control. In saline-sodic soils, nutrient imbalances and high pH restrict the availability of nitrogen, zinc and boron, thereby limiting leaf initiation and expansion (Karimi *et al*., 2005). The microbial consortium helped to overcome these constraints through nutrient solubilization and phytohormone production (cytokinins, IAA and gibberellins), which stimulated the cell division, shoot organogenesis and cell

elongation, ultimately increasing both leaf number and leaf surface. In addition, EPS production and antioxidant enzyme activation (catalase, SOD, peroxidase) improved water balance and reduced oxidative stress, supporting sustained leaf emergence and turgidity under salt stress conditions (Shah, 2016 and Sarkar *et al*., 2018).

Micronutrients delivered through EPNS and micronutrients mixture further contributed to leaf growth. Zinc enhanced auxin biosynthesis, boron strengthened cell walls and supported meristematic activity, while manganese and copper facilitated enzymatic functions and chloroplast development (Ali *et al*., 2011; Hussain *et al*., 2012 and Zaman *et al*., 2020). The synergistic action of the microbial consortium and micronutrient supplementation clearly outperformed RDF-only treatments, highlighting the importance of optimized nutrient formulations with biological delivery systems in improving canopy development and photosynthetic capacity under sodic conditions.

Yield Parameters of Rice

Grain and Straw Yield (q ha^{-1})

The application of halophilic microbial consortium (HM) and micronutrient-enriched EPNS significantly enhanced rice grain and straw yields under sodic soil conditions. Among the application methods, seed treatment combined with soil application (M3) produced the highest grain yield (47.41 q ha^{-1}) and straw yield (61.10 q ha^{-1}), followed by soil application alone and seed treatment (Table 6). With respect to nutrient sources, the HM + EPNS consortium (S2) resulted in the highest grain (45.08 q ha^{-1}) and straw (57.49 q ha^{-1}) yields. The interaction effect (M×S) showed that M3S2 (seed treatment + soil application of HM+EPNS) recorded the maximum yields (48.56 and 62.45 q ha^{-1}), closely followed by M3S3 (HM+MM consortium) with 48.12 and 62.34 q ha^{-1}. The least grain (35.34 q ha^{-1}) and straw (42.44 q ha^{-1}) yields were observed in the control.

The combined application of halophilic microbial consortium (HMC) with EPNS significantly improved nutrient availability, root development and biomass, leading to higher grain and straw yield. Halophilic microbes contributed higher grain and straw yields by producing phytohormones, ACC deaminase and exopolysaccharides, which enhanced cell proliferation, root growth and soil structure while reducing

Table 6

Grain and straw yield of rice as influenced by the application of expired pharmaceuticals and micronutrients enriched halophilic microbial consortium

Treatments	Grain yield (q ha^{-1})	Straw yield (q ha^{-1})
Methods of application		
M1	39.67	50.89
M2	43.53	55.69
M3	47.41	61.10
S.Em±	0.53	0.65
C.D @ 5 %	1.54	1.89
Source of nutrients		
S1	41.63	53.08
S2	45.08	57.49
S3	43.90	57.11
S.Em±	0.53	0.65
C.D @ 5 %	1.54	1.89
T_1 Control	35.34	42.44
S.Em±	0.90	1.11
C.D @ 5 %	2.70	3.20
Interaction (M×S)		
T_2 M1SI	37.12	47.42
T_3 M1S2	41.56	53.12
T_4 M1S3	40.32	52.12
T_5 M2S1	42.22	53.31
T_6 M2S2	45.12	56.90
T_7 M2S3	43.25	56.87
T_8 M3S1	45.56	58.52
T_9 M3S2	48.56	62.45
T_{10} M3S3	48.12	62.34
S.Em±	0.92	1.12
C.D @ 5 %	2.67	3.27

ethylene stress under sodic conditions (Glick *et al*., 2007 and Shrivastava & Kumar, 2015). Enrichment with micronutrients (Zn, Fe, Mn, Cu) and macronutrients (Ca, Mg, S) through EPNS further supported chlorophyll synthesis, photosynthesis and stress tolerance, ensuring better plant metabolism (Singh *et al*., 2009 and Rathod *et al*., 2019). These treatments also improved ionic homeostasis by lowering Na^+ accumulation and enhancing K^+ and Ca^{2+} uptake, resulting in favorable Na^+/K^+ and Na^+/Ca^{2+} ratios that sustained photosynthetic efficiency and biomass accumulation (Munns & Tester, 2008 and Yang *et al*., 2009). Overall, the synergy of microbial and nutrient enrichment, particularly *via* seed treatment and soil application, proved effective in overcoming sodicity-related limitations and offers a sustainable strategy for enhancing rice yield in saline soils.

No. of Panicle per Metre Square

EPNS enriched Halophilic Microbial Consortium with different methods and sources showed significant variation at harvest recording highest number of panicles per m^2 in seed treatment + soil application (270.2 m^2). With regard to sources of application HM + EPNS consortium (248.6 m^2) recorded highest number of panicles per m^2 is presented in Fig. 1.

The interactive effect of EPNS enriched halophilic microbial consortium with different methods and sources recorded higher number of panicles per m^2 T_9 (280.8 m^2) treatment that received seed treatment + soil application of HM + EPNS consortia which is on par with T_{10} (274.7 m^2) that received HM + MM consortium in the same manner. The lowest was recorded in control (T_1-176.7 m^2).

Per Cent Chaffiness

The chaffiness was significantly reduced with improved application methods with the lowest percentage observed in M3 (15.64%), followed by M2 and M1. Among the nutrient sources, HM+EPNS consortia (S2) recorded markedly lower chaffiness (17.66%) compared to the control (25.45%) (Fig. 1). Among the interactions T_9 (seed treatment + soil application of HM + EPNS) showing the lowest value (14.78%).

The combined application of halophilic microbial consortium (HM) with expired pharmaceutical nutrient supplements (EPNS) significantly increased the number of panicles per m² compared to the control, primarily due to the enhanced vegetative growth, greater tiller formation and improved nutrient uptake. The synergistic effect of microbes and

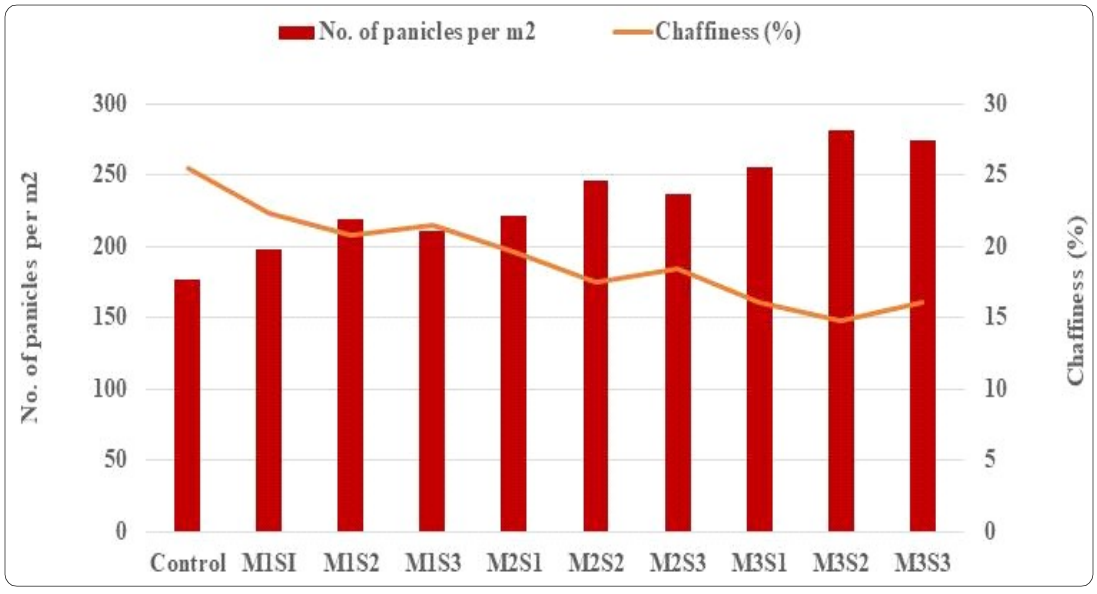

Fig. 1 : Effect of expired pharmaceuticals and micronutrients enriched halophilic microbial consortia on number of panicles per m^2 and % chaffiness of rice

micronutrients supported key metabolic processes such as auxin production, protein synthesis and efficient photosynthesis, which are vital for panicle initiation and elongation. Improved ion balance, particularly lower Na^+ /K^+ and Na^+ /Ca^{2+} ratios (Mohamed and Gomaa, 2012 and Prasad *et al*., 2018) further contributed to better panicle development and grain filling (Munns, 2005). Moreover, a notable reduction in per cent chaffiness was recorded under HM+EPNS treatments, reflecting improved pollen fertility and spikelet filling efficiency facilitated by micronutrients like boron and zinc (Suman & Sheeja, 2018 and Helio *et al*., 2019). The stress-alleviating mechanisms of halophilic microbes, including ACC deaminase activity, osmolyte production and antioxidant defence, also played a crucial role in sustaining reproductive development under sodic conditions. Together, these improvements highlight the effectiveness of integrating microbial consortium with micronutrient supplementation in optimizing panicle production and reducing chaffins in rice grown under salt stress conditions.

This study demonstrates that the integration of halophilic microbial consortium (HMC) enriched with expired pharmaceutical nutrient supplements (EPNS) constitutes a viable and sustainable strategy to enhance rice growth, yield and salinity tolerance under salt-affected conditions. The observed improvements could be attributed to the synergistic interplay between microbial activities and re-purposed nutritional inputs, which collectively enhanced the nutrient bioavailability, root system architecture and key physiological processes. The co-application of HMC with either EPNS or conventional micronutrient salts ensured a holistic nutrient management approach, delivering essential macro- and micronutrients while promoting ionic homeostasis and hormonal equilibrium. These microbial consortium significantly contributed to the improved stress resilience through modulation of rhizosphere dynamics and plant metabolic responses, thereby fostering optimal growth in saline environments. Moreover, the valorization of expired supplements not only addresses environmental concerns associated with pharmaceutical waste but also contributes to sustainable agriculture and global food security by improving crop productivity in marginal soils.

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Uncovering the Genetic Diversity of *Lysinibacillus sphaericus* Strains Isolated from Soil Ecosystems

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Abstract

Biocontrol agents play a pivotal role in sustainable agriculture by mitigating plant diseases through eco-friendly mechanisms that reduce reliance on chemical inputs. *Lysinibacillus sphaericus* is a gram-positive, ubiquitous, spore-forming bacterium well known for its insecticidal activity against dipterans, particularly mosquitoes. Yet, systematic investigation of native *Lysinibacillus sphaericus* strains from this region remains limited, particularly those exhibiting both entomolytic and plant growth-promoting traits. In this study, five indigenous *Lysinibacillus sphaericus* strains were isolated from soil collected across Karnataka. The isolated strains were screened based on morphology, Gram staining with terminal spherical endospores, biochemical and molecular characteristics. Molecular characterization using 16S rRNA gene sequencing showed 95-100 per cent homology with the reference strains and phylogenetic analysis grouped them within the *L. sphaericus* lineage. The strains were further assessed for their biocontrol and plant growth-promoting potential. All five strains exhibited protease activity, along with varying levels of lipase, lecithinase and chitinase expression, suggesting potential entomolytic properties. Key plant growth-promoting rhizobacterial traits such as indole-3-acetic acid production, ammonia generation and siderophore production were observed in the majority of the strains. Among the isolates, strain NBAIR_LS76 showed the highest potential across both functional categories. These findings highlight the potential of *L. sphaericus* as a biocontrol agent due to its dual application in pest control and sustainable crop production. The study underscores the significance of exploring unexplored ecosystems for microbial resources and identifies strain NBAIR_LS76 as a promising candidate for *in vivo* validation and bioformulation development in integrated nutrient and pest management in agriculture.

Keywords : *Lysinibacillus sphaericus*, 16S rRNA, Phylogenetic tree, Endospore, Crystal protein

Biological control agents play a pivotal role in sustainable disease and pest management by offering ecologically friendly alternatives to synthetic chemicals. These agents significantly enhance plant health functioning as a bio-stimulant, reduce effects on non-target organisms and natural ecosystems, while minimizing the residual risks and resistance issues associated with chemical alternatives (Prasannakumar *et al*., 2017 and Supriya *et al*., 2024). Among microbial biocontrol agent, such as *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma harzianum*, *Lysinibacillus sphaericus* (formerly *Bacillus sphaericus*) is particularly notable for its strong activity against dipteran pests, establishing it as an important tool in the management of vector-borne diseases (Ahmed *et al*., 2007).

Lysinibacillus sphaericus is a Gram-positive, mesophilic, rod-shaped, aerobic, spore-forming

bacterium ubiquitous in soil and aquatic ecosystems, notable for producing highly resistant endospores during sporulation, which can survive harsh environmental conditions and persist in diverse ecological niches (Ruqiya *et al*., 2023). It is well known for the production of different toxins like binary toxins (BinA/BinB), along with vegetative toxins like Mtx and Cry proteins, which, upon ingestion by insect larvae, are activated by midgut proteases, bind to specific epithelial receptors and induce pore formation, resulting in cell lysis and death (Silva-Filha *et al*., 2021).

In addition to biocontrol potential, several *L. sphaericus* strains have been recognized for improving plant health by producing plant growth-promoting traits like siderophores, indole-3-acetic acid and ammonia, triggering systemic resistance in plants by activating defence enzymes such as protease, lipase, lecithinase and chitinase, which play a pivotal role in sustainable agriculture (Riseh *et al*., 2024). Collectively, these enzymes deliver direct antagonism by degrading vital structural components of pests and pathogens. Moreover, they provide indirect benefits: enzymatic degradation products serve as elicitors of plant defense mechanisms and the improved root colonization by biocontrol agents enhances plant resilience and growth. The synergistic action of multiple enzymes with PGP activity often results in superior biocontrol efficacy compared to single activity approaches (Mejia *et al*., 2024). As eco-friendly alternatives to chemical pesticides, enzyme-producing biocontrol agents offer targeted, efficient and environmentally conscious strategies for crop protection.

Although *Lysinibacillus sphaericus* is recognized for its established applications, its broader potential in agriculture remains under explored. Emerging research suggests that certain strains possess plant growth-promoting rhizobacterial (PGPR) traits such as the production of phytohormones, ammonia and siderophores that can enhance soil fertility and boost crop productivity. These environments may harbor untapped microbial diversity with significant agricultural value. This study aims to fill that gap by isolating and characterizing indigenous *L. sphaericus* strains from soil samples collected at mosquito-infested sites in Karnataka. Through detailed morphological, biochemical and molecular analyses, as well as functional assays, the study evaluates their biocontrol activity and PGPR potential, aiming to identify promising strains for use as bioinoculants in integrated nutrient and pest management strategies.

Material and Methods

Collection of Soil Samples and Isolation of *Lysinibacillus sphaericus*

To explore the natural reservoirs of *Lysinibacillus sphaericus*, a total of 10 soil samples were aseptically collected from diverse regions in Karnataka (Fig. 1). Details of the sampling locations are provided in Table 1. The isolation of *L. sphaericus* from soil samples was carried out as per the protocol described by Ankitha *et al*. (2023). Specifically, 10 g of each soil sample was suspended in 100 mL of autoclaved distilled water and subjected to shaking incubation at 30°C and 150 rpm for 4 hours to facilitate the release of endospores and vegetative cells. Post incubation, 1 mL aliquots of the suspension were heat-treated at 80°C for 20 minutes to selectively eliminate gram-negative bacteria, ensuring the enrichment of

Fig. 1 : Geographical mapping of the different soil and insect samples collected from mosquito breeding sites of Karnataka

Table 1

Description of the soil samples collected for the isolation of *Lysinibacillus sphaericus*

Isolate name	Location	Geological coordinates
UASB_LS1	Bengaluru Urban, Karnataka	13.0968 ^{0}N, 77.5666 ^{0}E
UASB_LS2	Dakshina Kannada, Karnataka	12.9156 ^{0}N, 74.8560 ^{0}E
UASB_LS3	Mandya, Karnataka	12.5237 ^{0}N, 76.8947 ^{0}E
UASB_LS4	Tumakuru, Karnataka	13.341 ^{0}N, 77.1011 ^{0}E
UASB_LS5	Bengaluru Rural, Karnataka	13.1261 ^{0}N, 77.0042 ^{0}E
UASB_LS6	Hassan, Karnataka	13.0022 ^{0}N, 76.1044 ^{0}E
UASB_LS7	Davangere, Karnataka	14.4678 ^{0}N, 75.9209 ^{0}E
UASB_LS8	Chitradurga, Karnataka	14.2255 ^{0}N, 76.3978 ^{0}E
UASB_LS9	Chikkamagaluru, Karnataka	13.3196 ^{0}N, 75.7719 ^{0}E
UASB_LS10	Kolar, Karnataka	13.1357 ^{0}N, 78.1320 ^{0}E

endospore-formers like *L. sphaericus* (Ruqiya *et al*., 2024). Once cooled to room temperature, the treated suspensions were serially diluted and appropriate dilutions were plated onto Nutrient Yeast Salt Mineral (NYSM) agar. The NYSM medium was composed of nutrient agar (28 g/L), magnesium chloride ($MgCl_2.6H_2O$, 0.2 g), yeast extract (0.5 g), calcium chloride ($CaCl_2.2H_2O$, 0.1 g) and manganese chloride ($MnCl_2 \cdot 4H_2O$, 0.001 g), adjusted to pH 7.0. Plates were incubated at 28°C for 48 hours. Colonies with characteristic morphological features suggestive of *L. sphaericus* were picked and sub-cultured onto fresh NYSM agar (Fig. 2) (Prabakaran *et al*., 2007). Subcultures were incubated for 24 hours at 28°C and then maintained at 4°C for further analysis (Mythra *et al*., 2023).

Morphological and Molecular Characterization of *Lysinibacillus sphaericus*

Isolated colonies were subjected to morphological characterization, including assessment of colony shape, size, texture, opacity, elevation, margin and pigmentation after 24 hrs of inoculation. Cellular morphology, spore formation and Gram reaction were determined through microscopic examination using Gram staining procedures with observations which was taken under a light microscope after 2 days of inoculation (Olympus BX41, Pennsylvania, USA) (Pramesh *et al*., 2024 and Bharath *et al*., 2024).

To confirm the identity of the strains, Genomic DNA from all the strains was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The 16S rRNA gene was amplified *via* polymerase chain reaction (PCR) using universal bacterial primers FD2 (5'-AGAGTTTGATCCTGGCTCAG-3') and RP1 (5'-ACGGTTACCTTGTTACGACTT-3'). The PCR protocol was optimized using genomic DNA from NBAIR BSWG1 and specific primers. A 25µL reaction mixture was prepared, consisting of 12.5µL of 2X Emerald Amp GT PCR Master Mix (Takara, Japan), 1µL each of forward and reverse primers

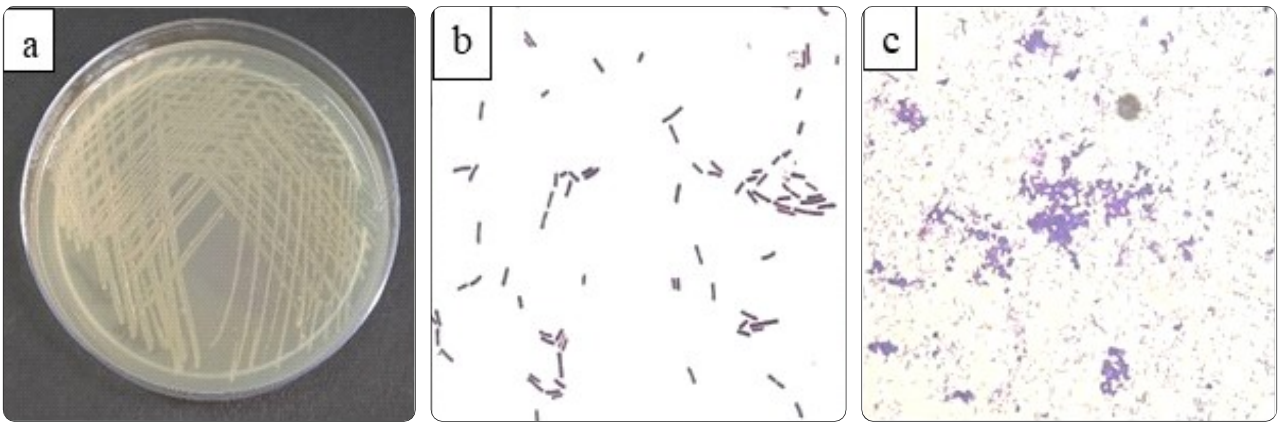

Fig. 2 : Morphological and microscopic characterization of strains. a) Pure culture showing off white coloured, round and raised colonies, b) Gram staining showing Gram-positive rod-shaped bacteria, c) Crystal protein staining

(10/ pmol/µL), 5.5 µL of molecular biology-grade water, and 5 µL of template DNA. Amplification was carried out in a thermal cycler (T100, Bio-Rad, California, USA) with the following conditions: initial denaturation at 95 °C for 3/ min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s and extension at 72 °C for 45 s; followed by a final extension at 72 °C for 5 min and a hold at 4 °C. PCR products were separated on a 1.5 per cent agarose gel and visualized using the MiniLumi gel documentation system (DNR, Israel) as reported by Ruqiya *et al.* (2022).

The PCR products were resolved by electrophoresis on a 1.5 per cent agarose gel and visualized using a gel documentation system (DNR MiniLumi, Israel). Amplicons were then subjected to Sanger sequencing (Ruqiya *et al*., 2023) and the resulting sequences were analyzed using BLASTn on the NCBI platform to determine similarity with known bacterial taxa. The validated sequences were deposited in GenBank and accession numbers were obtained. For phylogenetic analysis, sequence alignments and tree construction were performed**,** where nucleotide sequences were aligned using the Molecular Evolutionary Genetics Analysis software (MEGA) version 11.0. Multiple sequence alignment was carried out employing the ClustalW/MUSCLE algorithm. The aligned sequences were then used for phylogenetic tree construction using the Maximum Likelihood method. Evolutionary distances were computed using the Kimura 2-parameter model (Tamura *et al*., 2021). A consensus tree was generated from 1,000 bootstrap replicates to assess the robustness of phylogenetic groupings. Branches with bootstrap support values below 50 per cent were collapsed to improve interpretability (Kumar *et al*., 2018).

Screening for Plant Growth-promoting Traits

All bacterial strains were screened for essential plant growth-promoting (PGP) traits, including the production of indole-3-acetic acid (IAA), ammonia, and siderophores. To assess IAA production, bacterial strains were cultured in nutrient broth supplemented with 0.5 g/L of L-tryptophan, which serves as a precursor for IAA synthesis. The cultures were incubated under dark conditions at 28 ± 2 °C for 72 hours with continuous agitation at 120 rpm to ensure optimal growth. The cultures were centrifuged to obtain a cell-free supernatant. The supernatant was then mixed with Salkowski's reagent, which reacts with IAA to produce a pink coloration. Indole-3-acetic acid (IAA) production was quantified using the Salkowski colorimetric method. The bacterial isolate was grown in production medium (e.g., LB or minimal medium) supplemented with L-tryptophan (0.1-1.0 g L^{-1}) and incubated for 48-72 h at the appropriate temperature with shaking. After incubation, the cultures were centrifuged at 10,000 × g for 10 min, and the cell-free supernatant was collected. Freshly prepared Salkowski reagent was then added to the supernatant in a 1:2 ratio (1 mL supernatant + 2 mL reagent), mixed thoroughly and incubated at room temperature in the dark for 20-30 min to allow color development. The appearance of a pink coloration indicated IAA production. Absorbance was measured at ~530 nm using a spectrophotometer, with a reagent or medium blank serving as control. IAA concentration was determined from a standard calibration curve prepared with known concentrations of commercial IAA (0-100 µg mL^{-1}) processed under identical conditions and the results were expressed as µg IAA per mL of culture (Pantoja-Guerra *et al*., 2023). Ammonia production was assessed by inoculating the bacterial strains into buffered peptone water and incubating the cultures at 30 ± 0.1 °C for 72 hours. Following incubation, the presence of ammonia in the culture supernatant was determined (Cappuccino and Sherman, 2008). Siderophore production was assessed qualitatively using Chrome Azurol S (CAS) agar medium. Strains were spot-inoculated onto the CAS plates and incubated under standard conditions. The discolouration occurs around the colonies from blue to orange or yellow, indicating the production of siderophores, reflecting the isolate's ability to chelate iron from the medium (Schwyn and Neilands, 1987).

***In-vitro* Enzyme Activity Assays**

The enzymatic capabilities of *Lysinibacillus sphaericus* strains were investigated to understand their potential role in biological control. A total of 5 strains were subjected to enzyme activity screening, where a 10/ µL volume of the 24-hour broth culture was spot-inoculated, followed by incubation at 30 °C for 48 hours, after which halo formation was

assessed in all the strains. Protease activity was assessed using skim milk agar plates. After incubating the inoculated plates at 30 °C for 48 hours, zones of clearance indicated proteolytic activity (Shabanamol *et al*., 2021). Lipase production was evaluated using tributyrin agar. A 10 µL aliquot of the bacterial broth was spotted onto the agar surface and halo formation after 48 hours of incubation at 30 °C indicated lipolytic activity (Sumarsih *et al*., 2024). Lecithinase activity was detected using egg yolk agar, where the formation of opaque zones around the colonies signified enzyme production (Sharaf *et al*., 2014). Chitinase activity was tested on chitin agar containing 4 per cent crude chitin as the sole carbon source (Naureen *et al*., 2017).

Results and Discussion

Morphological and Molecular Characterization with Phylogenetic Analysis

In the present study, a total of 10 distinct bacterial morphotypes were initially isolated from soil samples collected across Karnataka and cultured on NYSM agar. Among these, five strains exhibited characteristic colony morphology typical of *Lysinibacillus sphaericus*, including circular, off-white colonies with flat elevation, rough surface texture, entire margins, and an opaque appearance (Fig. 3). Microscopic analysis further confirmed their identity, revealing rod-shaped, Gram-positive cells with terminal endospore formation (Fig. 3). All five strains tested positive for crystal protein production, a hallmark feature of *L. sphaericus*, consistent with the findings of Rocha *et al.* (2023). Comprehensive morphological features are summarized in Table 2. Taxonomic identification was further validated through 16S rRNA gene sequencing, which yielded amplicons of approximately 1,400-1,500 bp. BLASTn analysis revealed 95-100 per cent sequence similarity to known *L. sphaericus* strains in the GenBank database (Fig. 4a) and the corresponding accession numbers are listed in Table 3. Phylogenetic analysis, including the five isolates, one reference strain and six closely related species, revealed clustering into two distinct clades (Fig. 4b), reflecting known intra-species diversity within *L. sphaericus* (Felsenstein, 1985). This diversity may not only be associated with entomolytic properties but also suggests adaptive genomic and phenotypic variations linked to plant-beneficial traits and ecological niche specialization.

In vitro Plant Growth-Promoting Activity

All five *Lysinibacillus sphaericus* strains demonstrated a range of plant growth-promoting (PGP) traits, highlighting their potential as bioinoculants. Notably, all strains were capable of producing indole-3-acetic acid (IAA), a key

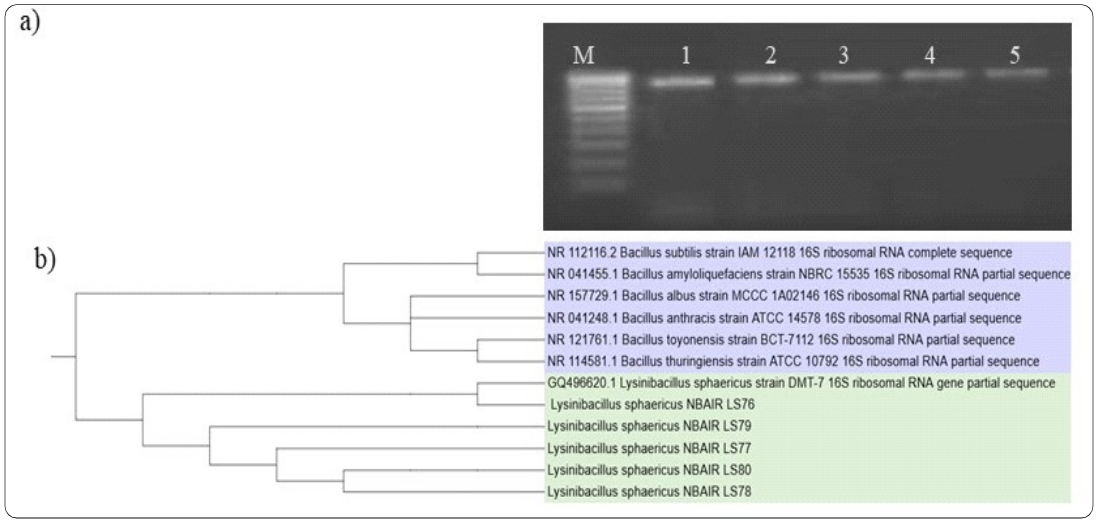

Fig. 3 : Molecular characterization of *Lysinibacillus sphaericus* strains. a) Agarose gel electrophoresis of a PCR amplification of the 16S rRNA gene from *Lysinibacillus sphaericus* strains. Lane 1-5: *Lysinibacillus sphaericus* NBAIR_LS76 to NBAIR_LS80. Lane M- ladder (100bp) Phylogenetic tree based on 16S rRNA gene sequences of *Lysinibacillus sphaericus* strains in circular representation. b) Phylogenetic tree which illustrates the clustering of the isolates using 16S rRNA gene sequences with known *Bacillus* species in rectangular representation

TABLE 2

Taxonomic characterization of *Lysinibacillus sphaericus* using morphological features

Isolate name	Colony shape	Colony morphology	Texture	Elevation	Margin	Colour	Opacity	Gram staining	Endospore staining
UASB_LS1	Circular	Rod-shaped	Rough	Flat	Entire	Off-White	Opaque	-	-
UASB_LS2	Circular	Rod-shaped	Smooth	Flat	Entire	Cream	Opaque	+	+
UASB_LS3	Circular	Rod-shaped	Smooth	Raised	Undulated	Cream	Opaque	+	-
UASB_LS4	Circular	Rod-shaped	Rough	Flat	Entire	Off-White	Opaque	+	+
UASB_LS5	Circular	Rod-shaped	Rough	Flat	Entire	Off-White	Opaque	-	-
UASB_LS6	Circular	Rod-shaped	Rough	Flat	Entire	Off-White	Opaque	+	-
UASB_LS7	Irregular	Rod-shaped	Smooth	Flat	Entire	Off-White	Opaque	+	+
UASB_LS8	Circular	Rod-shaped	Smooth	Flat	Undulated	Cream	Opaque	+	+
UASB_LS9	Circular	Rod-shaped	Rough	Raised	Entire	Off-White	Opaque	-	-
UASB_LS10	Circular	Rod-shaped	Smooth	Flat	Entire	Cream	Opaque	+	+

TABLE 3

Genotypic characterization of *Lysinibacillus sphaericus* strains with GenBank accession numbers

Isolate name	Strain name	Accession number
UASB_LS2	*Lysinibacillus sphaericus* NBAIR_LS76	PV878061
UASB_LS4	*Lysinibacillus sphaericus* NBAIR_LS77	PV878062
UASB_LS7	*Lysinibacillus sphaericus* NBAIR_LS78	PV878074
UASB_LS8	*Lysinibacillus sphaericus* NBAIR_LS79	PV878073
UASB_LS10	*Lysinibacillus sphaericus* NBAIR_LS80	PV878581

phytohormone involved in regulating root cell division and elongation, promoting lateral and adventitious root formation, improving nutrient uptake and stress resilience, especially vital under nutrient-deficient conditions (Kukreti *et al*., 2024a). IAA production was qualitatively confirmed by the appearance of a distinct pink coloration upon reaction with Salkowski's reagent, indicating the presence of indole compounds in the culture supernatant. This consistent IAA production across all strains underscores their potential role in enhancing plant growth and productivity (Fig. 5a). Indeed, IAA-producing *Lysinibacillus* strains have previously been shown to increase biomass and root growth in crops like corn (Naureen *et al*., 2017). In addition to IAA production, all strains were capable of generating ammonia, confirmed by the development of a yellow to orange coloration upon treatment with Nessler's reagent (Fig. 5b), which signifies a bioavailable nitrogen source with potential nitrogen-fixation implications and mitigating reliance on chemical fertilizers (Acevedo and Dussan, 2020). Additionally, three strains also demonstrated siderophore production through the appearance of orange halos on Chrome Azurol S (CAS) agar plates (Fig. 5c), which are powerful iron-chelating molecules, enhancing iron acquisition in iron-limited soils and suppressing pathogenic microorganisms by depriving them of this vital nutrient. Besides pathogen suppression, siderophores also facilitate iron uptake by plants, helping to alleviate iron deficiency in calcareous or nutrient-poor soils, thereby encouraging better growth and vigour (Al-Raish *et al*., 2025). Notably, strains NBAIR_LS76 and NBAIR_LS77 tested positive for all three PGP traits and exhibited the highest levels of IAA and ammonia production (Table 4). The overall correlation of PGP traits among the strains was represented through a heat map in Fig. 6.

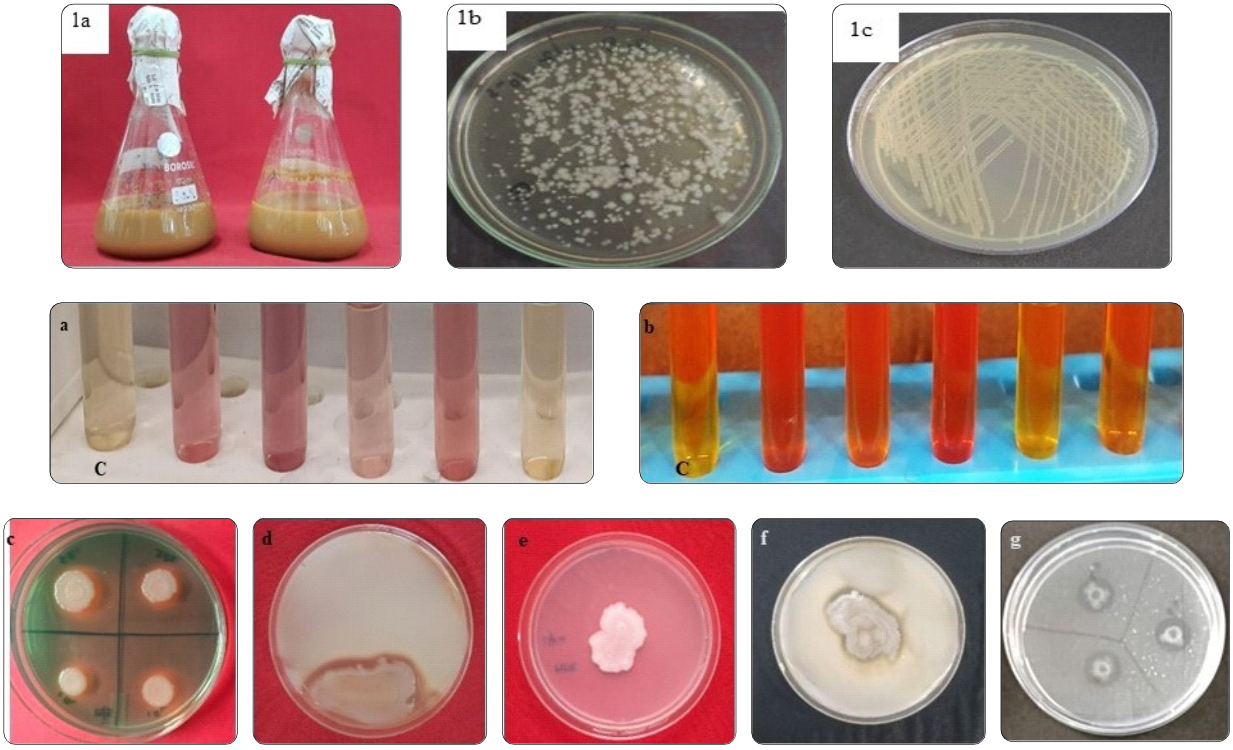

Fig. 4 : Biochemical characterization showing plant growth-promoting and enzyme production activities of *Lysinibacillus sphaericus* strains a) IAA production test showing pink-orange colour as positive, C- control, b) Ammonia production test showing yellow-brown colour as positive, C- control, c) Siderophore production showing yellow zone around the colony as positive in CAS agar, C- Control, d) Protease test showing clear zone around the colony as positive in starch casein media, e) Lipase test showing hallow zone around the colony as positive in tributyrin media, f) Lecithinase test showing opaque zone around the colony as positive in egg yolk agar media, g) Chitinase test showing hallow zone around the colony as positive in colloidal chitin agar media

TABLE 4

Assessment of PGP attributes and extracellular enzyme production by *Lysinibacillus sphaericus* strains

Strain name	Plant growth-promoting			Hydrolytic enzyme activity			
	IAA production	Ammonia production	Siderophore production	Protease activity	Lipase activity	Lecithinase activity	Chitinase activity
NBAIR_LS76	+	+	+	+	+	+	+
NBAIR_LS77	+	+	+	+	-	+	-
NBAIR_LS78	+	+	-	-	+	-	-
NBAIR_LS79	+	+	-	+	-	+	+
NBAIR_LS80	+	+	-	-	-	-	+

Collectively, these PGP traits contribute both directly and indirectly to enhance plant health by promoting better root architecture, increasing nutrient absorption, alleviating abiotic stress and offering protection against soil-borne pathogens (Manjunatha *et al*., 2019). Harnessing such multifunctional microbes enables the development of sustainable, eco-friendly agricultural systems.

In vitro Enzyme Activity

A critical factor in biocontrol effectiveness is the production of a diverse array of lytic enzymes,

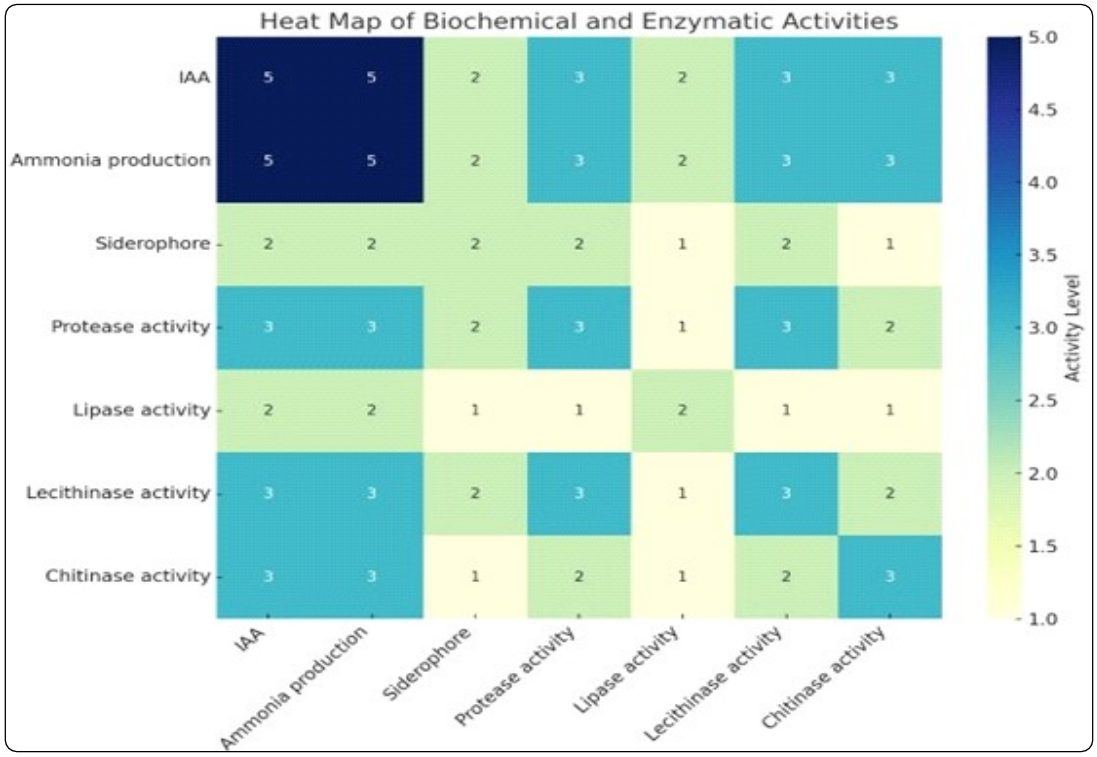

Fig. 5 : Heat map represents the levels of biochemical and enzymatic activities across different strains. Each cell indicates the number of strains exhibiting a particular activity, with colour intensity corresponding to the activity level

including protease, lipase, lecithinase and chitinase, which act as entomolytic and biocontrol agents in *L. sphaericus*. Protease activity was observed in three strains, marked by clear zones on skim milk agar plates. Proteases facilitate the breakdown of structural proteins in pathogen cell walls and insect cuticles, weakening their integrity and preventing development a phenomenon well documented among *Bacillus* and fungal biocontrol strains (Admassie *et al*., 2022) (Fig. 5d). Lipase activity was identified in two strains using tributyrin agar, shown by halo formation around the colonies (Fig. 5e). Lipases break down lipid layers in microbial membranes and insect exoskeletons, which aids microbial invasion and improves enzymatic penetration (Sumarsih *et al*., 2024). Moreover, lecithinase production was demonstrated by three strains through the formation of opaque zones on egg yolk agar (Fig. 5f). Lecithinases, acting as phospholipases targeting phosphatidylcholine, further disrupt pest cell membranes and work synergistically with other enzymes (Sharaf *et al*., 2014). Chitinase activity was confirmed in three strains by the appearance of clear halos on chitin agar plates (Fig. 5g). Chitinases specifically cleave β1,4-glycosidic bonds in chitin, a major component of fungal cell walls and exoskeletons, leading to physical collapse and pathogen death (Shabanamol *et al*., 2017). These enzymes are essential for nutrient cycling, host colonization and pathogen suppression in both soil and insect environments. Acting as key virulence factors, they help overcome host defenses and enhance the antagonistic potential of *L. sphaericus* against phytopathogens. Notably, strain NBAIR_LS76 exhibited the highest enzymatic activity across all tests, highlighting its potential as a biocontrol agent for managing phytopathogens.

Together, these traits underscore the multifaceted benefits offered by *L. sphaericus* strains that enhance plant nutrition, help plants resist disease and support overall plant vigour. While every isolate exhibited at least one plant growth-promoting trait, the specific combinations and intensities of traits varied markedly. The enzymatic profile provides valuable insight into the potential of *L. sphaericus* strains for use in biocontrol applications based on their ability to

degrade structural and storage components of fungal pathogens. Notably, NBAIR_LS76 isolated from the soil sample stood out for its superior hydrolytic enzyme activity alongside robust PGP properties. This discovery highlights the untapped potential of soil-associated *L. sphaericus* strains and supports their exploration for future biotechnological and sustainable agricultural applications.

In conclusion, this study successfully isolated and characterized indigenous *Lysinibacillus sphaericus* strains, revealing their promising potential for biocontrol applications and plant growth promotion. A total of 5 strains were comprehensively analysed, demonstrating considerable genetic and functional diversity shaped by their ecological origins. Notably, the strain NBAIR_LS76 exhibited superior lytic enzyme activity along with robust PGP traits, highlighting its value as a dual-purpose agent for sustainable agriculture. These findings position as a strong candidate for development into a bioinoculant capable of enhancing crop productivity while suppressing phytopathogens. To fully realize its potential, further studies involving toxin profiling, field trials and formulation development are essential. Genomic insights and real-world validation will be critical for integrating this strain into eco-friendly crop management and disease control strategies.

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Effect of Integrated Nutrient Management on Growth, Yield and Economics of Horsegram (*Macrotyloma uniflorum*)

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ABSTRACT

Horsegram (*Macrotyloma uniflorum*), a vital leguminous crop known for its resilience and nutritional value, faces yield limitations due to suboptimal nutrient management. Integrated Nutrient Management (INM) has emerged as a key strategy to enhance productivity and maintain soil health by combining organic, inorganic and biological inputs tailored to specific crop and soil needs. This study aims to evaluate the impact of different INM practices on horsegram agronomic performance and soil properties in Andhra Pradesh's semi-arid conditions. This experiment was conducted at Agricultural Research Station, Anantapuramu, Andhra Pradesh during 2023-24. Experiment was conducted in randomized complete block design with three replications and treatments combining organic amendments, mineral fertilizers and biofertilizers. The results demonstrated that INM practices significantly enhanced plant height, pod and seed yield and soil nutrient content. Treatment T4 (50 % Recommended Dose of Fertilizers + Rhizobium + Phosphorus Solubilizing Bacteria + 25% vermicompost) showed the higher performance across multiple parameters, including yield, nutrient uptake and economic returns. Economic returns were highest for treatment T4, highlighting its profitability and efficiency in nutrient management. INM practices, especially the combination of 50% RDF, Rhizobium, PSB and 25 per cent vermicompost, significantly enhanced horsegram productivity and soil health in terms of nutrients.

Keywords : Integrated nutrient management in horsegram, Nutrient uptake, Soil health, Yield

HORSEGRAM (*Macrotyloma uniflorum*), a leguminous crop, is renowned for its resilience in marginal environments and its nutritional value, making it an essential food source in many parts of Asia and Africa. Despite its importance, the potential yield and sustainability of horsegram cultivation are often constrained by suboptimal nutrient management practices. Integrated Nutrient Management (INM) has emerged as a promising strategy to enhance crop productivity while maintaining soil health. INM involves the judicious combination of organic, inorganic and biological inputs tailored to specific crop and soil requirements. Horsegram is predominantly cultivated in India with major producing states including Karnataka, Tamil Nadu, Andhra Pradesh and Odisha. As of recent estimates, horsegram is grown on approximately 1.2 million hectares globally with India accounting for over 70 per cent of the total area. In Andhra Pradesh, horsegram is cultivated on approximately 1,50,000 hectares. This region's diverse agro-climatic conditions support horsegram cultivation, particularly in rainfed areas. Global production of horsegram is estimated to be around 0.8 million metric tons with India producing approximately 0.6 million metric tons annually (Rai and Shivran, 2021). The annual production of horsegram in Andhra Pradesh is around 90,000 metric tons.

This substantial output highlights the crop's significance in the region's agriculture. The average productivity of horsegram varies significantly across different regions. In India, the national average yield is about 500-600 kg/ha. However, research and improved agronomic practices have shown that yields can potentially reach up to 900-1000 kg/ha under optimal conditions. The average productivity of horsegram in Andhra Pradesh is about 600 kg/ha. However, with improved agronomic practices and the adoption of INM, yields can potentially increase to 900-1000 kg/ha under optimal conditions. Long-term studies indicate that INM practices contribute to better soil health by increasing soil organic carbon (SOC), enhancing microbial activity and improving soil structure.

This study examines the effect of INM on the growth and yield in horsegram cultivation. The primary objective is to evaluate how different nutrient management practices influences the agronomic performance of horsegram and the underlying soil properties. By integrating organic amendments with mineral fertilizers and biofertilizers, this research aims to provide insights into sustainable agricultural practices that optimize resource use efficiency and promote long-term soil fertility. Given the growing interest in sustainable agriculture and the need to improve food security, this study's findings will contribute to the broader understanding of nutrient management in leguminous crops. It will also offer practical recommendations for farmers and policy makers aiming to enhance the productivity and sustainability of horsegram farming systems.

Material and Methods

The field experiment was conducted at the Agricultural Research Station (ARS) in Anantapuramu, Andhra Pradesh, India during 2023-24 to evaluate the effect of different nutrient management practices in Horsegram. The geographical coordinates of the site are approximately 14.68° N latitude and 77.60° E longitude. The climate of Anantapuramu is semi-arid, with hot summer and mild winter. The average annual rainfall is around 550 mm, most of which is received during the southwest monsoon season from June to September. The rainfall is highly variable and often insufficient to meet the crop water requirements, making irrigation a critical component of agricultural practices in this region. The region is characterized by a semi-arid climate with scarce and erratic rainfall. Meteorological data is presented in Fig. 1. The average maximum temperature over the recorded period was 32.95°C and the average minimum

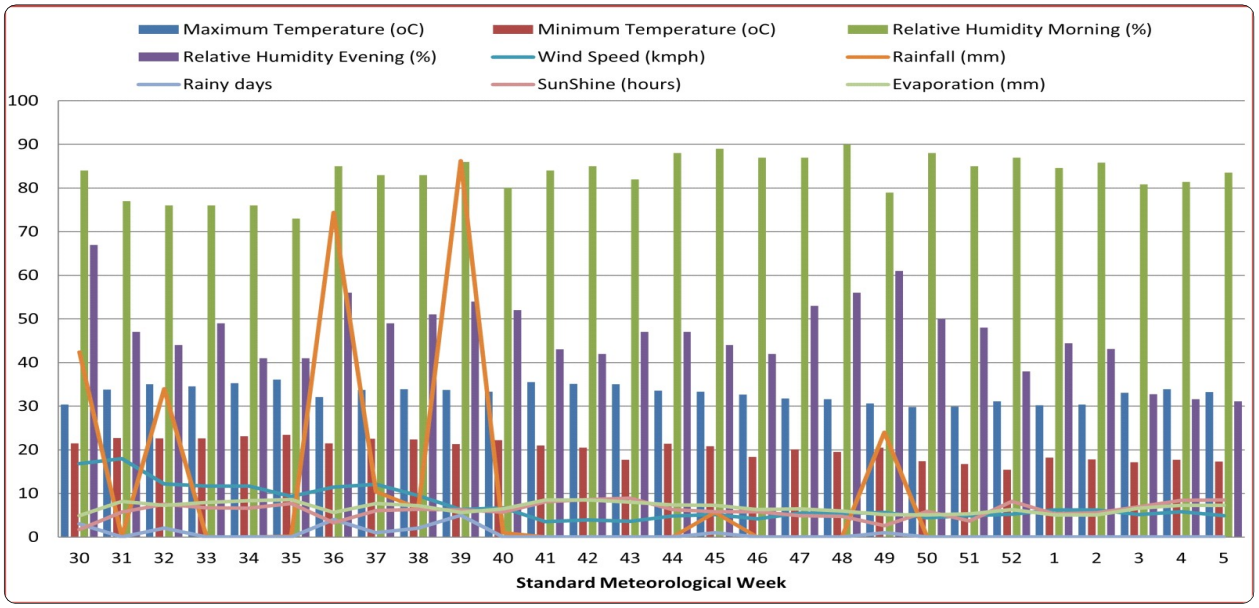

Fig. 1 : Weekly meteorological data recorded during the crop growth

temperature was 20.12°C. The average morning relative humidity was 83.08 per cent, while the average evening relative humidity was 46.61 per cent. The average wind speed was 7.50 kmph. The total rainfall recorded was 284.8 mm over 19 rainy days. The average sunshine hours per week were 6.13 hours, and the average evaporation rate was 6.75 mm per week.

The soil at the experimental site is classified as red sandy loam. Prior to the commencement of the study, soil samples were collected from the top 0-15 cm layer across the experimental plots. The samples were air-dried, ground and passed through a 2 mm sieve for analysis of physical and chemical properties. The soil was analyzed for pH, electrical conductivity, organic carbon, available nitrogen, phosphorus and potassium using standard soil testing procedures. Soils were slightly alkaline with pH of 7.97, EC of 0.07 with low organic carbon (0.09%), low nitrogen (212.9 kg/ha), low phosphorus (12.7 kg/ha), medium in potassium (294 kg/ha) and low micronutrients (Copper-0.08 ppm, Manganese-0.59 ppm, iron-0.43 ppm and zinc-0.50 ppm).

Crop was sown during September, 2023 and harvested during January, 2024. A randomized complete block design (RCBD) with three replications was employed for the study. The experimental plots measured 5 m x 4 m each. Treatments comprised of T_1: Control (100% RDF), T_2: 100% RDF + Foliar spray of urea @ 2%, T_3: 100% RDF + seed treatment with Rizobium + PSB, T_4: 100% RDF + seed treatment with Rizobium + PSB + 2% urea spray, T_5: 100% RDF + seed treatment with Rizobium + PSB + 1% 19:19:19 spray, T_6: 50% RDF + 2.5 t/ha FYM, T_7: 50% RDF + 2.5 t/ha FYM+ 2% urea spray, T_8: 50% RDF + 2.5 t/ha FYM + 1% 19:19:19 spray. ATPHG 11 was taken as test variety. Seeds were sown with spacing of 30 x 10 cm. Seed treatment was done with carbendazim @ 1 g/kg seed. Fertilizers were applied as per the treatment requirements. 100% RDF comprised of 4 kg of nitrogen, 10 kg of phosphorus and 8 kg potash supplying fertilizers which were broad casted before sowing. Seed treatment with Rizobium and PSB was done before sowing @ 10 ml/kg seed. Foliar spray of urea and 19:19:19 was done when crop was subjected to moisture stress. Weeds were controlled through the application of Pendimethalin @ 1.0 lt/ac one day after sowing and manual weeding was done twice at 20 and 40 DAS.

Data on growth parameters like plant population, plant height, number of branches/plant, plant fresh and dry weight were recorded before harvesting. At maturity, the yield parameters such as number of pods per plant, pod weight, seed yield per plot and 1000-seed weight were measured. Harvesting was done with sickles by cutting the plant to the ground level. Threshing was done by drying the harvested produce for 2 days on threshing floor and trampled with tractor.

Harvest Index (%) = Economic yield/Biological yield x 100 (Donald, 1962).

Where,

Economic yield = Seed yield

Biological yield = Seed yield + Bhusa yield

RWUE (kg hamm^{-1}) = Yield (kg ha^{-1})/ Total water use (mm) (Cheema *et al.,* 1991)

Production Efficiency (kg ha^{-1} day^{-1}) = Seed Yield (kg ha^{-1})/ Duration of the crop (days) (Tomar and Tiwari, 1990).

Nutrient uptake by Stover (kg ha^{-1}) = Nutrient concentration in stover (%) x Stover yield (q ha^{-1})

Crude Protein per cent = N content (%) x 6.25

Crude protein yield (q ha^{-1}) = Crude protein (%) x stover yield (q ha^{-1})/100

Gross return (Rs.ha^{-1}) = (Seed yield x price) + (bhusa yield x price)

Net returns (Rs.ha^{-1}) = Gross return (Rs.ha^{-1}) - Cost of cultivation (Rs.ha^{-1})

Benefit: Cost ratio = Gross returns (Rs.ha^{-1})/ cost of cultivation (Rs.ha^{-1})

The collected data were subjected to statistical analysis using SPSS. Analysis of variance (ANOVA) was performed to determine the significance of treatment effects. Means were compared using the Least Significant Difference (LSD) test at a 5 per cent probability level. The correlation matrix was created using R studio version 1.4.1717 utilizing the 'ggcorrplot' package. Economics was calculated based on the prevailing market price of inputs and labour wages. The input and output prices of the commodities prevailing during the study were taken into an account for calculating the net returns and benefit-cost ratio.

Results and Discussion

Growth Parameters

The growth parameters such as plant height, number of branches per plant varied significantly among the treatments (Table 1). The highest plant height was observed in T4 (93.3 cm), followed by T5 (89.7 cm), indicating that these treatments are beneficial for vertical growth. T4 also had the highest number of branches per plant (9.33), showing that the integrated nutrient management practices in this treatment promote branching. This suggests that a balanced approach using both organic and inorganic inputs promotes better vegetative growth in Horsegram. Treatments with only chemical fertilizers or lower levels of organic manure showed comparatively lower growth, emphasizing the importance of organic inputs for optimal growth. The positive effects of INM on plant growth can be supported by the work of Swetha *et al*. (2018) who found that combining organic and inorganic fertilizers improved the vegetative growth parameters of legumes by enhancing nutrient availability and uptake.

Yield Attributes and Yield

The yield attributes like pods per plant, seed yield, bhusa yield and harvest index were also influenced by the different treatments (Table 2). T4 had the highest number of pods per plant (98.7), suggesting enhanced reproductive performance under this treatment. The highest seed yield was recorded in T4 (958.7 kg/ha), followed closely by T5 (955.3 kg/ha). This indicates that these treatments are optimal for maximizing seed production. Bhusa yield, which includes the non-seed parts of the crop, was highest in T4 (1916.7 kg/ha), demonstrating the overall biomass increase with effective nutrient management. The harvest index was relatively consistent across treatments, with T6 showing a slightly higher value (36.57%), suggesting efficient conversion of biomass to economic yield. The treatment involving a combination of FYM,

Table 1

Growth parameters of Horsegram as influenced by integrated nutrient management practices

Treatments	Plant population (per sq.m)	Plant height (cm)	No. of branches/pt	Fresh plant weight (g)	Dry plant weight (g)
T_1	33	81.7	9	62.33	17.43
T_2	33	85.0	9	65.42	17.89
T_3	33	83.0	9	63.33	17.50
T_4	34	93.3	9	66.64	19.00
T_5	34	89.7	9	65.71	18.67
T_6	31	66.7	7	48.33	14.31
T_7	32	75.7	8	51.67	16.34
T_8	32	74.7	7	50.01	15.83
CD @ 5%	-	12.48	1.46	-	-
CV	-	8.77	9.92	-	-

Table 2

Yield attributes, yield and harvest index of horsegram as influenced by integrated nutrient management practices

Treatment	No. pods/ plant	No. of seeds/ pod	Pod length	100 fresh pod weight (g)	100 dry pod weight (g)	1000 fresh seed weight (g)	1000 dry seed weight (g)	Seed yield (kg/ha)	Bhusa yield (kg/ha)	Harvest index (%)
T_1	95.3	5.3	4.6	52.43	22.64	71.73	35.80	922.2	1896.7	32.71
T_2	98.0	5.3	4.7	55.40	23.84	75.00	36.80	952.0	1904.7	33.32
T_3	95.7	5.3	4.7	54.70	22.96	72.20	36.30	924.5	1898.1	32.78
T_4	98.7	5.3	4.9	56.13	24.44	76.67	37.87	958.7	1916.7	33.34
T_5	98.3	5.3	4.8	55.80	24.28	75.93	36.87	955.3	1910.0	33.34
T_6	81.0	5.0	4.2	43.67	19.68	64.40	34.27	761.4	1319.0	36.57
T_7	82.7	5.0	4.6	48.00	20.12	66.00	35.07	779.3	1582.9	33.23
T_8	82.3	5.0	4.4	47.90	19.84	65.23	34.50	776.1	1580.1	32.95
CD @ 5%	5.60	-	-	5.55	1.24	-	-	60.15	171.35	-
CV3.50	-	-	6.12	3.20	-	-	3.91	5.59	-	

chemical fertilizers and bio-fertilizers resulted in the highest number of pods per plant, seeds per pod and overall seed yield. This indicates that integrated nutrient management not only enhances growth parameters but also directly translates into higher yield. The increase in yield attributes with INM practices aligns with the findings of Verma and Mathur (2020), who reported that the application of biofertilizers along with chemical fertilizers significantly improved the yield and biomass of legumes. Higher seed and bhusa yields suggest that T4 not only enhances the main product but also the by-products, which are valuable as animal feed. Reddy *et al*. (2013) reported a 25 per cent increase in grain yield in legumes with the application of combined organic and inorganic fertilizers compared to sole applications. In horsegram, INM practices have been associated with a 30 per cent increase in biomass production and a 35 per cent increase in seed yield. A consistent harvest index suggests that the proportion of economic yield to total biomass remains stable, regardless of the INM practice used.

Water use and Production Efficiency

Water Use Efficiency (WUE) and Production Efficiency (PE) are crucial for understanding the resource use efficiency. T4 showed the highest WUE (4.39 kg/ha.mm), indicating efficient water utilization for biomass production. Similarly, T4 also had the highest PE (7.99 kg/ha/day), reflecting its effectiveness in converting growth inputs into yield (Table 3). Ghosh *et al*. (2019) demonstrated that INM practices improve water use efficiency in dryland

Table 3

Rain water use efficiency and production efficiency of Horsegram as influenced by integrated nutrient management practices

Treatments	Rain water use efficiency (Kg/ha.mm)	Production efficiency (kg/ha/day)
T_1	4.22	7.68
T_2	4.36	7.93
T_3	4.23	7.70
T_4	4.39	7.99
T_5	4.37	7.96
T_6	3.49	6.34
T_7	3.57	6.49
T_8	3.55	6.47
CD @ 5%	0.28	0.50
CV	3.91	3.91

crops by enhancing soil structure and moisture retention. Improved WUE and PE are critical in dryland agriculture, where water is a limiting factor. Ghosh *et al*. (2019) reported that INM practices enhance soil structure and moisture retention, leading to better water use efficiency .

Economic Analysis

Economic analysis helps in evaluating the profitability of different treatments. T2 had the highest net returns (Rs.19037/ha) despite not having the highest gross returns, indicating lower costs of cultivation relative to T4 and T5 (Table 4 and Fig. 2). T1 showed the highest benefit: cost ratio (3.11), meaning it provided the best return on investment. This highlights the profitability and cost-effectiveness of these treatments. High net returns and benefit: cost ratios suggest that certain INM practices can provide substantial economic benefits. Kumar *et al.* (2021) found that combining organic and inorganic fertilizers in chickpea cultivation resulted in higher net returns and benefit-cost ratios due to improved yields and reduced input costs. Prasad *et al*. (2019) indicated that farmers practicing INM in pulses experienced a 20 per cent increase in net returns compared to those using conventional methods.

Table 4

Economics of Horsegram as influenced by integrated nutrient management practices

Treat-ments	Cost of cultivation (Rs/ha)	Gross returns (Rs/ha)	Net returns (Rs/ha)	Benefit: cost ratio
T_1	8950	27796	18846	3.11
T_2	9525	28562	19037	3.00
T_3	9700	27859	18159	2.87
T_4	10275	28758	18483	2.80
T_5	10600	28657	18057	2.70
T_6	10950	22332	11382	2.04
T_7	11525	23441	11916	2.03
T_8	11850	23354	11504	1.97

Soil Health

Soil health indicators were monitored to assess the long-term sustainability of the treatments. Soil nutrient content varied with T5 showing the highest nitrogen

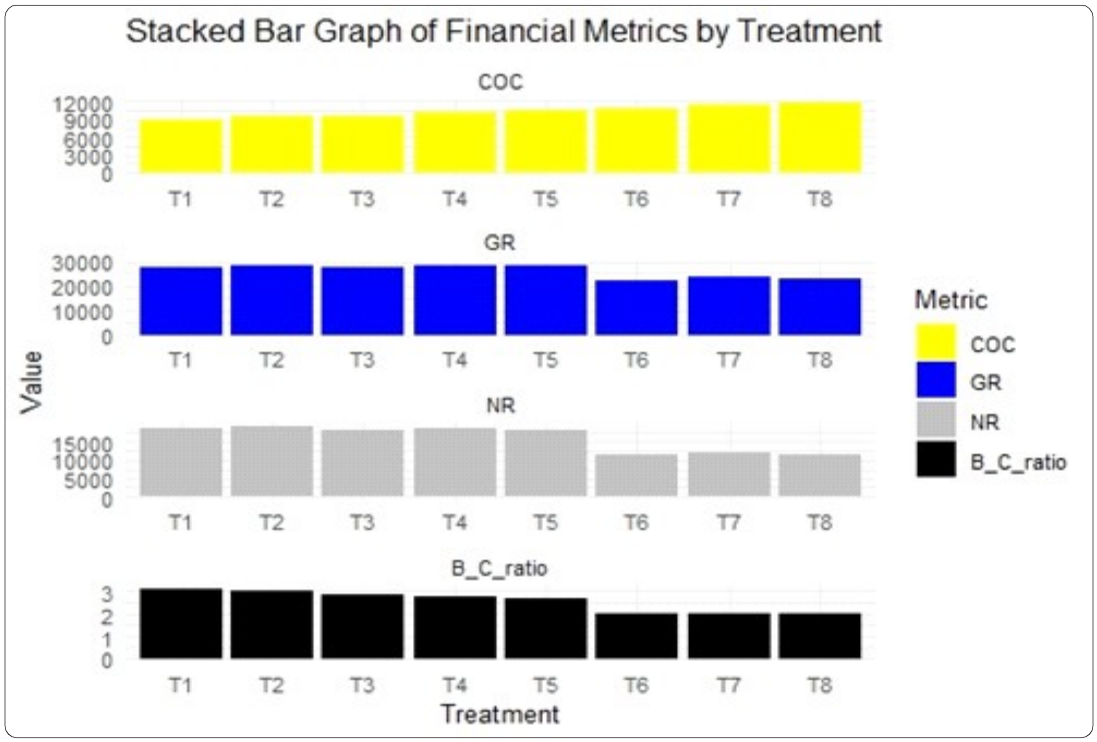

Fig. 2 : Stacked bar graph of economics

(265.6 kg/ha), T3 showing highest phosphorus (13.9 kg/ha), T2 showing highest potassium (265 kg/ha). (Table 5). Treatments did not significantly alter soil pH and organic carbon, suggesting the amendments were neutral in their effect on these properties. Fig. 3 and Fig. 4, revealed that Nitrogen content was increased in all treatments compared to initial. Whereas, potassium content was reduced in all treatments. Among micronutrients, iron content was reduced in all treatments compared to initial. Sharma and Saini (2017) reported that integrated nutrient management practices significantly enhance soil fertility by increasing the availability of macro and micronutrients and maintaining soil organic

TABLE 5

Effect of integrated nutrient management practices on soil physical properties, macro and micronutrients in Horsegram

Treatment	pH	EC	OC	N (kg/ha)	P (kg/ha)	K (kg/ha)	Cu (ppm)	Mn (ppm)	Fe (ppm)	Zn (ppm)
T_1	7.97	0.07	0.07	248.0	13.4	255	0.08	0.57	0.43	0.40
T_2	8.10	0.09	0.07	250.5	13.4	265	0.08	0.50	0.39	0.24
T_3	8.29	0.13	0.08	263.1	13.9	254	0.07	0.58	0.42	0.25
T_4	8.21	0.09	0.07	263.1	13.4	257	0.07	0.49	0.32	0.27
T_5	7.94	0.1	0.10	265.6	13.5	252	0.08	0.58	0.46	0.43
T_6	7.63	0.06	0.10	225.4	12.3	241	0.10	0.58	0.66	0.30
T_7	8.35	0.1	0.10	228.0	12.6	263	0.10	0.60	0.52	0.46
T_8	8.01	0.1	0.07	225.0	12.3	251	0.09	0.59	0.61	0.43
Initial	7.97	0.07	0.09	212.9	12.7	294	0.08	0.59	0.43	0.50
CD @ 5%	0.17	0.01	0.01	17.22	0.28	21.42	0.01	0.02	0.02	0.02
CV	1.23	4.15	4.29	4.00	1.22	4.80	4.58	1.58	1.86	3.08

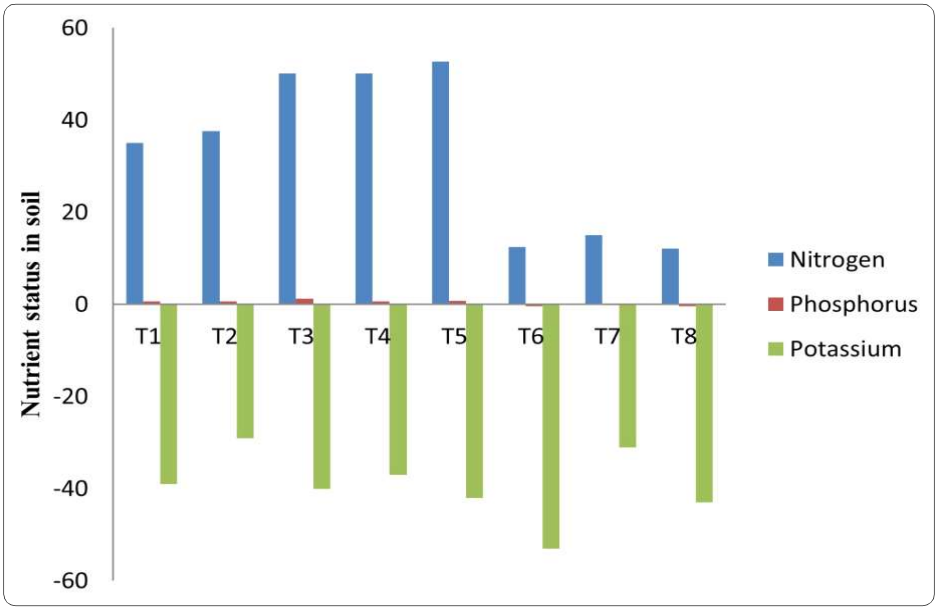

Fig. 3 : Macronutrient status of soil after harvest of the crop

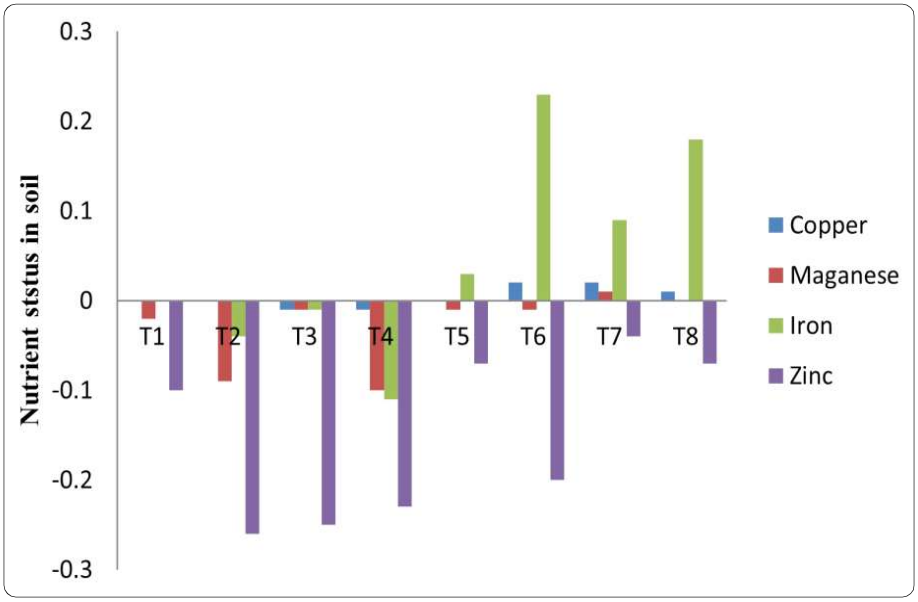

Fig. 4 : Micronutrient status in soil after harvest of the crop

carbon levels. Ravikumar and Suresh (2020) demonstrated that greengram plots treated with a combination of 50% RDF + FYM + Rhizobium inoculation produced significantly higher yields and improved soil organic carbon compared to chemical fertilizer alone. Manjunath and Chandrashekara (2019) reported that soil nutrient status (NPK) and pigeonpea yield was enhanced with the integrated application of 75% RDF + compost + biofertilizers, suggesting INM as a sustainable alternative to full chemical fertilization.

Nutrient uptake and Protein Content

The uptake of essential nutrients and crude protein content were significantly influenced by INM practices. T4 had the highest nitrogen (N) uptake (47.73 kg/ha), phosphorus (P) uptake (0.58 kg/ha) and potassium (K) uptake (28.18 kg/ha), indicating

TABLE 6

Effect of integrated nutrient management practices on nutrient uptake, crude protein per cent and crude protein yield of Horsegram

Treatment	N (kg/ha)	P (kg/ha)	K (kg/ha)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Crude protein %	Crude protein yield (q/ha)
T_1	41.35	0.57	20.48	0.04	0.10	0.59	0.05	13.61	2.58
T_2	44.57	0.57	21.71	0.04	0.10	0.66	0.03	14.63	2.79
T_3	44.04	0.57	26.38	0.03	0.19	0.84	0.03	14.53	2.75
T_4	47.73	0.58	28.18	0.03	0.19	1.03	0.06	15.58	2.98
T_5	44.89	0.57	32.28	0.02	0.25	1.24	0.06	14.69	2.81
T_6	26.91	0.40	6.99	0.07	0.28	1.48	0.10	12.83	1.68
T_7	34.19	0.47	12.50	0.08	0.34	1.59	0.11	13.76	2.14
T_8	33.18	0.47	15.01	0.06	0.26	1.36	0.12	13.17	2.07
CD @ 5%	1.80	0.03	2.93	0.01	0.01	0.08	0.01	1.42	0.11
CV	2.59	3.38	8.07	6.81	1.47	4.33	4.50	5.74	2.59

its superior nutrient utilization (Table 6). The crude protein content was highest in T4 (15.58%), resulting in the highest crude protein yield (2.98 q/ha), demonstrating improved nutritional quality of the crop. Jat and Ahlawat (2018) observed that the application of biofertilizers along with chemical fertilizers increased nutrient uptake and protein content in pulses, due to enhanced microbial activity and nutrient availability. Studies have shown that combining organic and inorganic fertilizers can improve soil structure, increase nutrient availability and enhance microbial activity, leading to better crop yields (Bhatt *et al*., 2019). The synergistic effect of organic manure and chemical fertilizers helps in the sustained release of nutrients, thereby supporting both vegetative and reproductive growth stages (Kumar *et al*., 2020).

Meteorological Data Impact on Yield of Horsegram

The meteorological data provided for the growing season shows a variation in weather conditions, which likely influenced the growth and yield of Horsegram (Fig. 1).

Rainfall : Significant rainfall events were recorded in certain weeks (*e.g.,* 11.6 mm on 10/08, 22.4 mm on 11/08). Adequate rainfall during the vegetative and flowering stages would have provided sufficient water for growth, especially in treatments with higher nutrient levels which typically require more water. Consistent periods without rainfall, particularly during later weeks, might have stressed plants in treatments without adequate soil moisture conservation practices like those receiving only chemical fertilizers.

Temperature : Maximum and minimum temperatures ranged from 27.2°C to 37.6°C and 18.2°C to 24.0°C, respectively. The temperatures were within the optimal range for Horsegram growth, supporting robust plant development. Variations in temperature, particularly high temperatures, could have influenced evapo transpiration rates and consequently, the water needs of the plants.

Relative Humidity : Morning relative humidity was relatively high (73% - 94%), which would have favored the vegetative growth and reduced water stress during early morning hours. Evening relative humidity showed more variation, impacting the transpiration rates and possibly the water use efficiency of different treatments.

Sunshine Hours : Adequate sunshine hours (ranging from 0.0 to 10.5 hours per day) were recorded, with higher sunshine promoting photosynthesis and plant growth. Weeks with lower sunshine hours, particularly in periods with high cloud cover and rainfall, could have temporarily reduced the photosynthetic activity, affecting growth rates.

Wind Speed : Wind speeds varied from 2.7 km/hr to 16.7 km/hr. Higher wind speeds can enhance transpiration rates, which may necessitate higher water uptake, particularly in nutrient-rich soils.

Adequate rainfall and optimal temperatures are crucial for legume crops. Excessive rainfall or drought can significantly affect flowering and pod filling stages (Patil *et al*., 2018). Consistent high relative humidity can reduce water stress and improve plant health, while extreme variations can stress the plants, impacting yield (Singh *et al*., 2017).

Overall, the integrated nutrient management practices significantly impacted the growth, yield, soil health and economic returns of Horsegram. Among the treatments, T4 (50% RDF + Rhizobium + PSB + 25% vermicompost) consistently showed superior performance across various parameters, making it the most effective INM strategy for enhancing both productivity and sustainability of Horsegram cultivation. Integrated nutrient management practices can significantly improve growth, yield, water use efficiency, economic returns, soil health and nutrient uptake in legumes such as Horsegram. By integrating organic and inorganic sources of nutrients, these practices ensure sustainable crop production while maintaining soil fertility. Further research could focus on long-term effects of these practices on soil health

and productivity, as well as their applicability to other crops and agro-climatic conditions.

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Agri Start-ups Ecosystem in Karnataka: Analysis and Challenges

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Abstract

Start-ups are increasingly becoming an important force for achieving social goals, applying technological innovations and creating new economic opportunities. In recent years, agritech start-ups have gained special attention and are often described as a 'ray of hope' for Indian agriculture. These start-ups focus on providing innovative solutions in areas such as precision farming, supply chain management and market linkages, which help farmers improve productivity, reduce costs and enhance their income. The present study was conducted during 2023-25 in Dharwad, Bengaluru, and Mysuru districts by selecting 30 Start-ups in agriculture and allied sectors from each district, comprising a total sample of 90 Start-ups. The results showed that a 43.33 per cent of the Start-ups were young, being less than three years old. Most of them were located in urban areas (77.77%) and nearly 78 per cent were at the scaling stage. About 40.00 per cent of the Start-ups belonged to the agritech sector and more than half (52.22%) offered advisory services. Among Government schemes, RKVY-RAFTAAR and ELEVATE-100 were the most commonly availed across all regions. Digital marketing, social media and accounting and legal compliance were the major outsourced activities, while sales, business development, product formulation and branding were largely handled in-house. Strong market demand and scalable sales were considered essential by many start-ups. Market expansion, customer acquisition and a focus on urban and niche consumer segments were the key growth strategies adopted. However, start-ups faced several challenges, particularly in acquiring early customers and securing funds. Climate change, environmental risks and market price volatility were also other major concerns. The findings highlight the need for stronger early-stage support, improved access to finance and better awareness of private funding options such as angel investors and venture capitalists. Overall, supportive policies, improved infrastructure and stronger ecosystem linkages are essential to promote sustainable growth of agritech start-ups across regions.

Keywords : Start-ups, Agriculture & allied sectors, Profile of Agri startups, Portfolio, Scaling-up

Agriculture and related industries are unquestionably India's primary source of income, particularly in its vast rural areas. People, big businesses and Start-ups are drawn to favourable soil and weather conditions, rising food demand, untapped potential, raw materials, manufacturing infrastructure, accessible credit programmes and a variety of Government incentives for marketing and export promotion. Agriculture is examined as an entrepreneurial endeavour that invests heavily in R & D, innovation, inventionand other business facets.

The term Start-up denotes the early stage of an enterprise integrating the science-based innovations

that can scale it up. Start-ups are emerging as a vehicle for realizing social purposes, a means of actualizing technological prowess, the wings of creative imagination that transports the purposeful dreamers to a land of their creation and preferred routes to riches. In recent years, there has been a surge of Agritech Start-ups, which are being termed as the 'Ray of Hope' in Indian agriculture. Agritech Start-ups are developing innovative solutions for various aspects of agriculture, including precision farming, supply chain management and market linkages.

Ecosystem of the Start-ups is operationally defined as a dynamic network of interconnected entities, organizations and resources that create the conducive environment for the growth and development of the Start-ups in agriculture and allied sectors and the factors affecting it. It also includes the portfolio and profile, Scaling-up aspects, Outsourcing and insourcing practices of the Start-ups along with the Challenges and risks associated with the Start-ups in agriculture and allied sectors. This ecosystem of the Start-up has evolved to encompass sectors like e-commerce and hyperlocal, influenced by factors such as swift urbanization, diverse diets, shifting consumer tastes and the growth of food markets.

Approximately 60.00 per cent of Agritech Start-ups are concentrated in Tier I & II cities within a select few states. In 2019, Agritech Start-ups raised over Rs. 1,840 crores (US$ 250 million) in venture funding, tripling the amount raised in 2018. Forecasts suggest that sector will attract over Rs.3,680 crores (US$ 500 million) in the coming years. In 2020, more than 20 Agritech Start-ups collectively secured over Rs.920 crores (US$ 125 million) through equity, venture debt and conventional debt rounds. Consequently, India's agritech ecosystem is poised for substantial expansion. Currently, three major trends dominate the industry: increased investments in agri-tech, a surge in digital literacy in Tier 2/ and Tier 3 cities and the rise of Farming-as-a-Service (FAAS) (Srinivasa *et al*., 2024).

India now being one of the world's hub of 'Start-up' ecosystem, ranking third with 90,000 'Start-ups'. Among the states of India, Karnataka is positioned at 14th place in the Global Start-up Ecosystem Report (GSER), Start-up Genome, 2025 [Anonymous, 2025 (a)]. According to the report given by government [Anonymous, 2025 (b)], it is one of the leading states with 16335 Start-ups after Maharashtra (27459) and followed by Delhi (15851).

There are 1,708 agriculture Start-ups in various fields of agriculture & allied sectors in India as of 2023-24. The top three states with the maximum number of agriculture Start-ups in India are Maharashtra with 226 Start-ups, Karnataka with 211 Start-ups followed by Tamil Nadu with 137 Start-ups (Anonymous, 2024). With the sector's ongoing expansion, it has the capacity to not only revolutionize India's agricultural landscape but also generate new job prospects and aid in the advancement of the nation's economy. As we move forward, it is important for Start-ups in agriculture and allied sector to stay focused on their mission of self-reliance and sustainability, building on the successes of the past. The vision of a self-reliant India is deeply embedded in the Start-ups ecosystem and will remain a guiding force for the years to come.

With this background present study was conceptualized with the follows specific objectives

To study the factors affecting the Start-ups in agriculture and allied sectors

To understand the challenges and risks faced by the Start-ups in agriculture and allied sectors

METHODOLOGY

The study was conducted during 2023-2025 in Dharwad, Bengaluru and Mysuru districts of Karnataka. From each district 30 Start-ups were randomly selected which comprised of 90 in total. *Ex-post facto* research design was followed and sampling was done through random sampling method. The data was collected by a pretested interview schedule by personal interview method. The data was analyzed and quantified using frequency,

percentage, Pearson correlation and multiple regression analysis.

Results and Discussion

Profile Characteristics of the Start-ups in Agriculture and Allied Sector

In the Table 1, it is clear that majority (40.00%) of the Start-ups from Dharwad region were of between 3 to 5 years, 60.00 per cent from urban region and had private limited type of ownership each. In Bengaluru Start-ups large proportion of them *i.e.,* 70.00 per cent were less than three years, 90.00 per cent of them were from urban areas and had private limited type of ownership each. Start-ups from Mysuru (36.67%) were of less than three years, 83.36 per cent were from urban areas, private limited type of ownership (53.34%). Dharwad and Bengaluru based Start-ups were having Agritech as the major sector of the Start-up along with Farmers as major customer segment, while majority of the Mysuru based Start-ups had Food related Start-ups with Consumers segment. Start-ups in Dharwad had 6-10 employees majorly, Bengaluru Start-ups (11-15 employees) and Mysuru Start-ups (less than 5 employees). The results in the study conducted by Aneesha (2021) and Shailesh (2022) also reflected the same findings.

As these three regions are the Start-up hubs of the Karnataka, the ecosystem of Start-up always encourages the new Start-ups emerging with innovative solutions, services and products to cater to the need of customers. The concentration of Start-ups in Urban areas across all three regions reflect the superiority of the facilities offered in the Urban region such as reliable internet connectivity, transport networks and access to co-working spaces, incubators and academic institutions (educational universities), which are essential for the functioning and growth of Start-ups. The customer segmentation patterns across the three regions showed how differently Start-ups in the agriculture and allied sectors connect with their markets. In Dharwad region, most of the Start-ups were from Agritech sector which serve farmers directly, exhibiting their focus on providing agriculture related technologies. Traders and consumers also form a significant part of the customer base, which suggests that Start-ups in Dharwad region are involved in both upstream (farm-level) and downstream (market-level) activities. Similarly, Bengaluru region focused on farmers and agritech related services. Mysuru region shows the strong presence of food processing Start-ups by catering majority of the general consumers. The higher presence of Start-ups with fewer skilled employees indicates a stronger orientation towards value-added

Table 1

Profile of the Start-ups in Agriculture and Allied Sectors

(n=90)

Characteristics	Category	Dharwad (n_1=30)		Bengaluru (n_2=30)		Mysuru (n_3=30)		Pooled (n=90)		Chi-square test Value
		f	%	f	%	f	%	f	%	
Age of the Start-up (in Years)	<3	07	23.33	21	70.00	11	36.67	39	43.33	9.51 *
	3-5	12	40.00	02	06.67	09	30.00	23	25.56	
	>5	11	36.67	07	23.33	10	33.33	28	31.11	
Location of the Start-up	Rural	05	16.67	00	00.00	01	03.33	06	06.67	6.45 NS
	Peri-urban	07	23.33	03	10.00	04	13.33	14	15.55	
	Urban	18	60.00	27	90.00	25	83.36	70	77.77	
Type of Ownership	Sole proprietorship	05	16.67	00	00.00	09	30.00	14	15.56	13.61 *
	General Partnership	03	10.00	00	00.00	01	03.33	04	04.45	
	Private Limited	18	60.00	27	90.00	16	53.34	61	67.77	

Continued....

Table 1 Continued....

(n=90)

Characteristics	Category		Dharwad (n_1=30) f	Dharwad %	Bengaluru (n_2=30) f	Bengaluru %	Mysuru (n_3=30) f	Mysuru %	Pooled (n=90) f	Pooled %	Chi-square test Value
	Limited Liability Partnership		04	13.33	03	10.00	04	13.33	11	12.22	
Stage of the Start-up	Ideation		02	06.67	01	03.33	01	03.33	04	04.44	7.42 NS
	Validation		03	10.00	02	06.67	00	00.00	05	05.56	
	Early traction		01	03.33	07	23.31	03	10.00	11	12.22	
	Scaling		24	80.00	20	66.67	26	86.67	70	77.78	
Sector of the Start-up	Agritech		18	60.00	12	40.00	06	20.00	36	40.00	10.35 NS
	Food processing		03	10.00	04	13.34	15	50.00	22	24.45	
	Agri-Biotech		04	13.34	01	03.33	00	00.00	05	05.55	
	Dairy		03	10.00	01	03.33	01	03.33	05	05.55	
	Organic		01	03.33	06	20.00	01	03.33	08	08.89	
	Others (Horticulture, Export, Handloom, Packaging)		01	03.33	06	20.00	07	23.34	14	15.56	
Customer Segmentation#	Farmers		19	63.33	20	66.66	06	20.00	45	50.00	8.65 NS
	Consumers		13	43.33	13	43.33	25	83.33	51	56.66	
	Traders		15	50.00	18	60.00	17	56.66	50	55.55	
Employees (in Numbers)	<5		07	23.33	02	06.66	12	40.00	21	23.33	15.13 NS
	6-10		13	43.33	05	16.67	08	26.66	26	28.89	
	11-15		06	20.00	07	23.33	05	16.67	18	20.00	
	15-20		01	03.33	05	16.67	01	03.33	07	07.78	
	>20		03	10.00	11	36.66	04	13.33	18	20.00	
Type of employment generated# (in numbers)	Skilled	<3	10	33.33	04	13.34	16	53.33	30	33.33	10.38 *
		4-10	16	53.33	13	43.33	10	33.33	39	43.34	
		>10	04	13.34	13	43.33	04	13.34	21	23.33	
	Technical	<2	23	76.66	12	40.00	21	70.00	56	62.22	9.39 NS
		3-5	04	13.33	09	30.00	06	20.00	46	21.11	
		>5	03	10.00	09	30.00	03	10.00	15	16.67	
	Administrative	<2	25	83.33	18	60.00	21	70.00	64	71.10	8.56 NS
		3-5	04	13.33	11	36.67	09	30.00	24	26.66	
		>5	01	03.33	01	03.33	00	00.00	02	02.22	
	Unskilled	Nil	20	66.67	17	56.67	27	90.00	64	71.10	7.16 NS
		1-2	08	26.67	10	33.33	03	10.00	21	23.33	
		>3	02	06.66	03	10.00	00	00.00	05	05.57	

#-Multiple responses; NS-Non-Significant; *-Significant at 5%

product units, micro-processing enterprises and consumer-focused agri-businesses that rely more on technical and administrative personnel rather than large, specialized skilled teams.

Portfolio of the Start-ups in Agriculture and Allied Sectors

A great majority of Dharwad Start-ups provided advisory services (53.33 %) and training & capacity-

building services (46.67%), emphasizing knowledge dissemination and skill enhancement along with Precision agriculture (63.33%) and targeted rural market (36.67%). The Bengaluru Start-ups services led by advisory services (63.33%) and training & capacity-building (56.67%), shows the city's strength in professional consultancy and mentoring ecosystems, agricultural inputs (50.00%) and targeted national markets (40.00%). Around 40.00 per cent of Start-ups of Mysuru region provided both training & capacity-building and advisory services, Value added food products (60.00%) and targeted national markets (36.66%).

The service offerings of Start-ups across all three regions show a clear focus on knowledge-based support, especially through advisory and training services. Since most ventures operate in agritech and food tech, guiding farmers and customers on new technologies has become essential. The overall

TABLE 2

Portfolio of the Start-ups in Agriculture and Allied Sectors

(n=90)

Characteristics	Category	Dharwad (n_1=30)		Bengaluru (n_2=30)		Mysuru (n_3=30)		Pooled (n=90)		Chi-square test Value
		f	%	f	%	f	%	f	%	
Services Portfolio#	Training and Capacity building services	14	46.67	17	56.67	12	40.00	43	47.77	20.14 NS
	Advisory services	16	53.33	19	63.33	12	40.00	47	52.22	
	Digitalized Services	03	10.00	00	00.00	03	10.00	06	06.67	
	Export Services	02	06.66	01	03.33	00	00.00	03	03.33	
	E-commerce platforms	03	10.00	00	00.00	00	00.00	05	05.55	
Products Portfolio#	Precision Agriculture & Equipment	19	63.33	06	20.00	06	20.00	31	34.44	21.62 NS
	Agricultural Inputs	02	06.66	15	50.00	02	06.66	19	21.11	
	Processed/ Value added food Products Manufacturing	02	06.66	08	26.66	18	60.00	28	31.11	
	Organic Agriculture products	01	03.33	02	06.66	05	16.66	08	08.89	
	Mobile Applications	02	06.66	01	03.33	01	03.33	04	04.44	
	Waste-to-Value Products	02	06.66	00	00.00	00	00.00	03	03.33	
	Others (Agri commodities selling, plant based cosmetics, ancient games, ecofriendly cups)	03	03.33	00	00.00	03	03.33	06	06.66	
Patents	Yes	09	30.00	20	66.67	15	50.00	44	48.89	7.89 *
	No	21	70.00	10	33.33	15	50.00	46	51.11	
Target Market	Rural	11	36.67	08	26.66	02	06.66	21	23.33	18.56 *
	Urban	04	13.33	01	03.33	00	00.00	05	05.57	
	National	05	16.66	12	40.00	16	53.33	33	36.66	
	Foreign	10	33.33	09	30.00	12	40.00	31	34.44	

TABLE 3

Economic Profile of the Start-ups in Agriculture and Allied Sectors

(n=90)

Characteristics	Category	Dharwad (n_1=30)		Bengaluru (n_2=30)		Mysuru (n_3=30)		Pooled (n=90)		Chi-square test Value
		f	%	f	%	f	%	f	%	
Capital Investment (Mean=6371000.00 SD=12383613.89)	Low (<Rs.179193.06)	04	13.33	02	06.67	03	10.00	09	10.00	9.36 NS
	Medium (Rs. 179193.06-Rs. 12562806.94)	25	83.33	21	70.00	24	80.00	70	77.77	
	High (>Rs. 12562806.94)	01	03.33	07	23.33	03	10.00	11	12.22	
Institutional Financial Sources#	Incubators	08	26.66	08	26.66	02	06.66	18	20.00	13.72 *
	Banks & Support	01	03.33	03	10.00	03	10.00	07	07.77	
	Government Grants	25	83.33	20	66.66	16	53.33	61	68.88	
	Venture Capitalists	00	00.00	07	23.33	01	03.33	08	08.88	
Non-Institutional Financial Sources#	Bootstrapping	29	96.66	25	83.33	30	100.00	84	93.32	5.98 NS
	Family & Friends	03	10.00	00	00.00	03	10.00	06	06.66	
	Angel Investors	00	00.00	03	10.00	02	06.66	05	05.56	
Institutional Financial Sources (Mean=1.06 SD=0.81)	Low (<0.65)	12	40.00	08	26.67	04	13.33	24	26.67	11.27 *
	Medium (0.65-1.46)	14	46.66	08	26.67	18	60.00	40	44.44	
	High (>1.46)	04	13.33	14	46.66	08	26.67	26	28.89	
Non-Institutional Financial Sources (Mean=1.06 SD=0.38)	Low (<0.87)	01	03.33	03	10.00	00	00.00	04	04.44	7.82 NS
	Medium (0.87-1.24)	26	86.66	26	86.66	25	83.33	77	85.55	
	High (>1.24)	03	10.00	01	03.33	05	16.66	09	10.00	
Funding Stages	Pre-Seed Funding	07	23.33	09	30.00	08	26.67	23	25.55	10.08 *
	Seed Funding	18	60.00	11	36.66	09	30.00	37	41.11	
	Series B Funding	00	00.00	01	03.33	00	00.00	01	01.11	
Annual income (Mean=15196944.44 SD=23410270.13)	Low (<Rs.3491809.38)	18	60.00	14	46.66	13	43.34	45	50.00	11.67 *
	Medium (Rs.3491809.38-Rs.26902079.51)	05	16.67	07	23.33	14	46.66	26	28.89	
	High (>Rs.26902079.51)	07	23.33	09	30.00	03	10.00	19	21.09	

product distribution clearly aligns with the sectoral and customer patterns and most Start-ups are strongly agriculture and farmer focused. The dominance of Precision Agriculture and Equipment shows the region's commitment to improving farm efficiency through mechanization, smart tools and technology-driven solutions. Bengaluru, however, displays a more varied product mix. The presence of organic products and precision tools adds to a balanced yet consumer-oriented product environment.

The reasons for consumers preferring organic products might be due to the factors like safety, nutrition, eco-friendly and taste (Naveena and Arunkumar, 2016). The market reach patterns show how Start-ups across Karnataka are expanding their presence beyond local boundaries. The smaller share of rural and urban markets suggests a clear shift from localized operations toward broader regional, national and global expansion. Because of the digitalization, easy transportation facilities and networking, the market penetration of Start-ups into different regions is taking place and as the districts selected for the research study are the Start-up hubs of Karnataka gives us the glimpse of market targets and their shifts.

Economic Profile of The Start-ups in Agriculture and Allied Enterprises

For the Dharwad region-based Start-ups, a great majority (80.00%) of the Start-ups reported a medium level of capital investment ranging between Rs.1,79,193.06 and Rs.12562806.94, while 13.33 per cent had low and 06.66 per cent had high investment levels. In Bengaluru, a similar pattern was observed, with 70.00 per cent of Start-ups operating under the medium investment range, 23.33 per cent under high investment and only 06.67 per cent in the low category. Among Mysuru-based Start-ups, 80.00 per cent reported medium investment, followed by 10.00 per cent of each with high and low investment levels, showing moderate capital deployment across ventures. When pooled, a significant proportion (76.66%) of Start-ups across all regions had medium-level investment, while 13.32 per cent had high and 10.00 per cent had low investment. Majority of the Start-ups across all the regions were availing financial assistance from Government grants (Institutional source) and Bootstrapping (Non-institutional source). Among Dharwad-based Start-ups, three-fifth of them had low level of annual income, 46.66 per cent of Bengaluru Start-ups had low level and in Mysuru, there was almost equal share between the medium and low level of annual income *i.e.,* 46.66 per cent and 43.33 per cent, respectively. When all the Start-ups across three regions were considered, half of them were having low level of annual income followed by other half distributed into medium (25.55%) and high (24.44%) levels of annual income. Majority of the results were found to be non-significant as Start-ups across all the regions shown similar trends with no much difference. Similar results were reported by Vipin (2022).

Different Schemes Availed by the Start-ups

The bird eye view of Table 4 shows the different schemes availed by the Start-ups. Three-fourth of the

TABLE 4

Different Schemes Availed by the Start-ups

(n=90)

Category	Dharwad (n_1=30)		Bengaluru (n_2=30)		Mysuru (n_3=30)		Pooled (n=90)	
	f	%	f	%	f	%	f	%
RKVY-RAFTAAR	18	60.00	06	20.00	05	16.67	29	32.22
DST-NidhiPrayas	02	06.67	02	06.67	04	13.33	17	18.89
ELEVATE-100	02	06.67	03	10.00	03	10.00	18	20.00
INCUBATORS	01	03.33	03	10.00	00	00.00	04	04.44
OTHERS (SISFS,MUDRA, PUSA KRISHI, BIRAC)	04	13.33	07	23.33	07	23.33	18	20.00
Not Availed	05	16.67	19	63.33	15	50.00	39	43.33

#-Multiple responses

Dharwad Start-ups (60.00%) benefited from the RKVY-RAFTAAR scheme under Krishik Agri business Incubator, UAS, Dharwad. One-fifth of the Bengaluru Start-ups benefitted from RKVY-RAFTAAR, followed by ELEVATE-100 and Incubators, 10.00 per cent each. Nearly One-fourth of the Start-ups (23.33%) availed benefits from other schemes whereas 16.67 per centbenefitted from RKVY-RAFTAAR. Half of the Start-ups from Mysuru, 63.33 per cent of Bengaluru Start-ups and 16.67 per cent of the Dharwad Start-ups and 43.33 per cent of overall Start-ups did not avail any of the schemes. Benefits availed by Start-ups across all three regions, under different schemes were like RKVY-RAFTAAR scheme, (32.22%) followed by ELEVATE 100 (20.00%) and Others schemes (20.00%). The results indicated that the uptake of Government schemes among Start-ups varies across regions, reflecting differences in awareness, accessibility and local ecosystem support. Dharwad Start-ups benefitted most from the RKVY-RAFTAAR scheme, likely due to the active role of Krishi Agri-Business Incubator at UAS, Dharwad in promoting and facilitating participation. In Bengaluru region, Start-ups accessed multiple schemes such as RKVY-RAFTAAR and ELEVATE-100, reflecting greater awareness, exposure to incubation networks and a more diverse Start-up ecosystem.

Mysuru Start-ups showed moderate participation, possibly because of limited information or fewer regional initiatives directly promoting these schemes. The relatively high proportion of Start-ups not availing any schemes indicates that there is lack awareness about the schemes available and difficulty in getting funds due to the political influences or any other recommendations. Many Start-ups opinioned that the expert committee which is set for scrutinizing the Start-ups for the fund release were of different background not from agricultural field. This lack of knowledge in both Start-ups and funders create a gap where many Start-ups were not able to get the required financial support. Overall, these patterns suggest that regional support systems, institutional outreach and knowledge about schemes strongly influence the extent to which Start-ups leverage government programs for growth and development.

Different Outsourcing and Insourcing Practices Followed by the Start-ups

Table 5 depicted the outsourcing and insourcing practices followed by the Start-ups. Software and app development were the major outsourced practice among Dharwad Start-ups, while for Bengaluru and Mysuru Start-ups major outsourced practices were digital marketing & social media (46.67 %) and accounting & legal compliance (46.67%), respectively. When the Start-ups across all the regions were considered, there was equal share of 33. 33 per cent between digital marketing & social media and accounting & legal compliance majorly followed by Supply chain & logistics and Software & app development with 22.22 per cent each. Product formulation and Branding was the highest insource practice for Dharwad Start-ups (96.66%) and Mysuru Start-ups (100.00%). Sales and Business Development was the highest insource practice for Bengaluru (93.32%) and Mysuru Start-ups (100.00%). When combined, Sales and Business development was highest with 95.55 per cent insources practice followed by Product formulation and Branding (91.10%).

The outsourcing and insourcing practices observed across the Start-ups reflect their strategic priorities and gaps in internal capabilities. Start-ups tend to outsource tasks that require specialized expertise, such as software development in Dharwad region, digital marketing in Bengaluru and accounting or legal compliance in Mysuru. This allows them to access professional skills without incurring the cost of hiring full-time experts. At the pooled level, digital marketing and regulatory tasks emerge as common outsourced functions because they demand technical knowledge and constant updates. In contrast, Start-ups strongly prefer to insource core functions that shape their identity and revenue, such as product formulation, branding, sales and business

TABLE 5

Outsourcing and Insourcing Practices Followed by the Start-ups

(n=90)

Outsourcing#	Dharwad (n_1=30)		Bengaluru (n_2=30)		Mysuru (n_3=30)		Pooled (n=90)	
	f	%	f	%	f	%	f	%
Supply Chain and Logistics	04	13.33	09	30.00	07	23.33	20	22.22
Digital Marketing and Social Media	04	13.33	14	46.67	12	40.00	30	33.33
Accounting and Legal Compliance	07	23.33	09	30.00	14	46.67	30	33.33
Software and App Development	09	30.00	09	30.00	02	06.66	20	22.22
Customer Support and Call Centers	00	00.00	01	03.33	00	00.00	01	01.11
Testing and Quality Assurance	03	10.00	04	13.33	03	10.00	10	11.11
Insourcing#								
Core Research and Development	16	53.33	22	73.33	21	70.00	59	65.55
Product Formulation and Branding	09	96.66	23	76.66	30	100.00	82	91.10
Sales and Business Development	28	93.32	28	93.32	30	100.00	86	95.55
Data Analytics and Precision Farming Insights	10	33.33	14	46.66	12	40.00	36	40.00
Relationship Management with Customers and Partners	21	70.00	17	56.66	22	73.33	60	66.66
Sustainability and Compliance Strategy	12	40.00	12	40.00	12	40.00	36	40.00

#-Multiple responses

development. These activities require direct founder involvement and close control to maintain quality and customer trust. Overall, the patterns suggest that Start-ups strategically balance efficiency and ownership by outsourcing specialized or time-consuming tasks while retaining the critical, value-creating processes within the organization.

Scaling-ups Aspects of Start-ups

Pre-requisites and Strategies for business Scaling-ups by the Start-ups are presented in Table 6. Sales and marketing (63.33%) followed by Strong market demand (40.00%) were the major pre-requisites for scaling up according to Dharwad Start-ups. Consistent revenue growth (46.66%) and access to adequate capital (36.68%) were the prerequisites for Bengaluru Start-ups. Strong market demand (70.00%), product-market fit (56.67%) and sales & marketing (56.67%) were the Mysuru based Start-ups pre-requisites for scaling up. Whereas, for the Start-ups across all the regions, strong market demand (52.22%), sales and marketing (52.22%), consistent revenue growth (40.00%) and product market fit (40.00%) were the major pre-requisites when put together. Market expansion and customer acquisition was the major strategy to scale -up the business followed by Dharwad (63.33%), Bengaluru (83.33%) and Mysuru (80.00%) and Overall (75.55%) Start-ups. Leveraging technology and Innovation was the next major strategy for 50.00 per cent of Bengaluru, while Strengthening supply chain and operations was for Dharwad (53.33%), Mysuru Start-ups (63.33 %) and Overall (52.22%) Start-ups.

Tapping into new markets required major strategies like leverage technology and digital platforms by Bengaluru Start-ups (76.66%), focusing on urban and niche consumer segments by Dharwad Start-ups (53.33%) and Mysuru Start-ups (70.00%) and Overall Start-ups (62.22%). For 63.33 per cent of Bengaluru Start-ups focusing on Urban and niche consumer

TABLE 6

Pre-requisites and Strategies to Scale-Up Business

(n=90)

Prerequisites for Scaling-up#	Dharwad (n_1=30)		Bengaluru (n_2=30)		Mysuru (n_3=30)		Pooled (n=90)	
	f	%	f	%	f	%	f	%
Consistent Revenue Growth	07	23.33	14	46.66	15	50.00	36	40.00
Strong Market Demand	12	40.00	10	33.33	21	70.00	47	52.22
Product-Market Fit	09	30.00	06	20.00	17	56.67	36	40.00
Access to Capital	01	03.33	11	36.67	04	13.33	11	12.22
Scalable Sales and Marketing	19	63.33	00	00.00	17	56.67	47	52.22
Reliable Infrastructure	00	00.00	00	00.00	03	10.00	03	03.33
Strategies to Scale-up the Business								
Market Expansion and Customer Acquisition	19	63.33	25	83.33	24	80.00	68	75.55
Technology and Innovation	08	26.66	15	50.00	15	50.00	38	42.22
Strengthening Supply Chain and Operations	16	53.33	12	40.00	19	63.33	47	52.22
Financial Growth and Funding Strategies	02	06.66	11	36.66	08	26.66	21	23.33
Brand Building and Customer Retention	12	40.00	07	23.33	13	43.33	32	35.55
Strategies to tap into new markets								
Leverage Technology and Digital Platforms	14	46.66	23	76.66	17	56.66	54	60.00
Focus on Urban and Niche Consumer Segments	16	53.33	19	63.33	21	70.00	56	62.22
B2B Partnerships and Institutional Sales	01	03.33	03	10.00	04	13.33	08	08.89
Innovate in Allied Sectors	00	00.00	00	00.00	02	06.66	02	02.22
Target Export and International Markets	10	33.33	08	26.66	04	13.33	22	24.44
Incentivize Farmer Adoption and Rural Expansion	15	50.00	07	23.33	11	36.67	33	36.66
Sustainability and Climate-Smart Agriculture	12	13.33	04	13.33	04	13.33	04	13.33

segments was second major strategy, while for 46.66 per cent of Dharwad and 56.66 per cent of Mysuru Start-ups and 60.00 per cent of Overall Start-ups second major strategy was leveraging technology and digital platform.

The results show that Start-ups across regions recognize different pre-requisites for scaling up based on their ecosystem maturity, market exposure and operational strengths. Dharwad Start-ups prioritize sales and marketing and strong market demand because many of them are still in early stages, where customer acquisition and visibility are critical for growth. Bengaluru Start-ups, being relatively more established, emphasize consistent revenue growth and access to capital, reflecting their focus on structured scaling, investor readiness and financial stability. Mysuru Start-ups highlight strong market demand and product-market fit, suggesting that they rely heavily on validating their solutions and ensuring steady customer acceptance before expanding. When strategies are considered, all regions over whelmingly adopt market expansion and customer acquisition as their primary approach, showing a common intention to widen reach and increase sales. Dharwad and Mysuru Start-ups additionally prioritize strengthening their supply chains, likely because efficient operations are

essential to support scaling in growing regional markets. Bengaluru's strong inclination toward leveraging technology and innovation reflects its advanced Start-up ecosystem and digital orientation. For tapping into new markets, Dharwad and Mysuru Start-ups focus on urban and niche consumer segments due to greater growth potential outside their local markets, whereas Bengaluru relies heavily on technology and digital platforms to expand faster. Overall, these patterns indicate that Start-ups choose prerequisites and strategies based on their stage of growth, available ecosystem support and the competitive dynamics of their operating environment.

Challenges and Risks Associated in Building the Start-ups

Results in the Table 7 clearly depicts the challenges and risks associated in building the Start-ups. Half of the Dharwad Start-ups faced a challenge in acquiring early customers followed by validating the idea (23.33%). For Bengaluru Start-ups, the challenge was about securing funding (56.67%) and building the right team (43.33%). Start-ups in Mysuru also faced a challenge in acquiring early customers followed by securing funding (70.00%). Overall, more than half of the Start-ups had challenges in acquiring early customers (55.55%) and securing funding (53.33%).Primary risks associated with Dharwad and Bengaluru Start-ups were climate change and environmental risks with 73.33 per cent and 83.33 per cent, respectively. Whereas, for Mysuru region it was market and price volatility (76.66%) closely followed by climate change and environmental risks (73.33%). When pooled, majority of the Start-ups foreseen Climate change and environmental risks (76.67%) followed by market and price volatility (70.00%) as challenges. Similar findings like challenges regarding secured funding

Table 7

Challenges and risks Associated in Building the Start-ups

(n=90)

Challenges faced in transitioning Idea into Start-up	Dharwad (n_1=30)		Bengaluru (n_2=30)		Mysuru (n_3=30)		Pooled (n=90)	
	f	%	f	%	f	%	f	%
Validating the Idea	07	23.33	05	16.67	13	43.33	25	27.77
Building the Right Team	02	06.66	13	43.33	05	16.67	20	22.22
Securing Funding	10	33.33	17	56.67	21	70.00	48	53.33
Developing an MVP	06	20.00	06	20.00	10	33.33	22	24.44
Acquiring Early Customers	15	50.00	12	40.00	26	76.66	50	55.55
Managing Uncertainty	00	00.00	00	00.00	03	10.00	03	03.33
Risks associated								
Climate Change and Environmental Risks	22	73.33	25	83.33	22	73.33	69	76.66
Market and Price Volatility	18	60.00	22	73.33	23	76.66	63	70.00
Supply Chain Disruptions	07	23.33	09	30.00	17	56.66	33	36.66
Financial and Funding Risks	08	26.67	13	43.33	19	63.33	40	44.44
Policy and Regulatory Risks	03	10.00	03	10.00	02	06.66	08	08.89
Technological and Cybersecurity Risks	00	00.00	01	03.33	02	06.66	03	03.33
Labor and Workforce Challenges	16	53.33	12	40.00	09	30.00	37	41.11
Consumer Behavior and Health Trends	06	20.00	07	23.33	09	30.00	22	24.44

#- Multiple responses

from institutional sources and skilled labours were reported by Rakshita *et al*. (2023).

The results indicate that Start-ups across the three regions face different challenges depending on their stage of development and ecosystem maturity. Dharwad and Mysuru Start-ups struggle most with acquiring early customers, which is common in emerging ecosystems where market awareness, customer trust and brand visibility are still developing. Dharwad's secondary challenge idea validation suggests that many founders lack access to expert feedback, pilot opportunities or market testing environments. Bengaluru Start-ups, operating in a more competitive and mature environment, report securing funding and building the right team as major challenges, reflecting high investor expectations and the constant demand for specialized talent in the city. At the overall level, acquiring early customers and securing funding emerge as the biggest obstacles, highlighting the dual struggle of gaining initial traction while also convincing investors of long-term potential.

In terms of risks, climate change and environmental uncertainty are major concerns for Dharwad and Bengaluru Start-ups, likely because many ventures operate in agri-related sectors where unpredictable weather, water scarcity and crop failures directly impact business operations. Mysuru Start-ups show higher concern for market and price volatility, possibly due to their dependence on fluctuating local markets and variable consumer purchasing power.

When combined, the dominance of climate-related risks and market volatility underscores the vulnerability of Start-upsespecially agriculture and allied sectorsto external shocks, unstable demand patterns and environmental disruptions. This reflects the need for robust risk management strategies, diversification and better access to predictive technologies to minimize uncertainties in their growth journey.

Core Concept of Farming-as-a-Service (FAAS)

Fig. 1, indicates the FAAS model of the Start-ups. FAAS offers technology-enabled farming services to farmers on a pay-as-you-go, subscription or freemium basis, helping reduce upfront investment and operational risks. Services include precision farming, data-driven advisory, supply chain access, equipment and input rentals and financial tools like credit and insurance embedded in the service package.

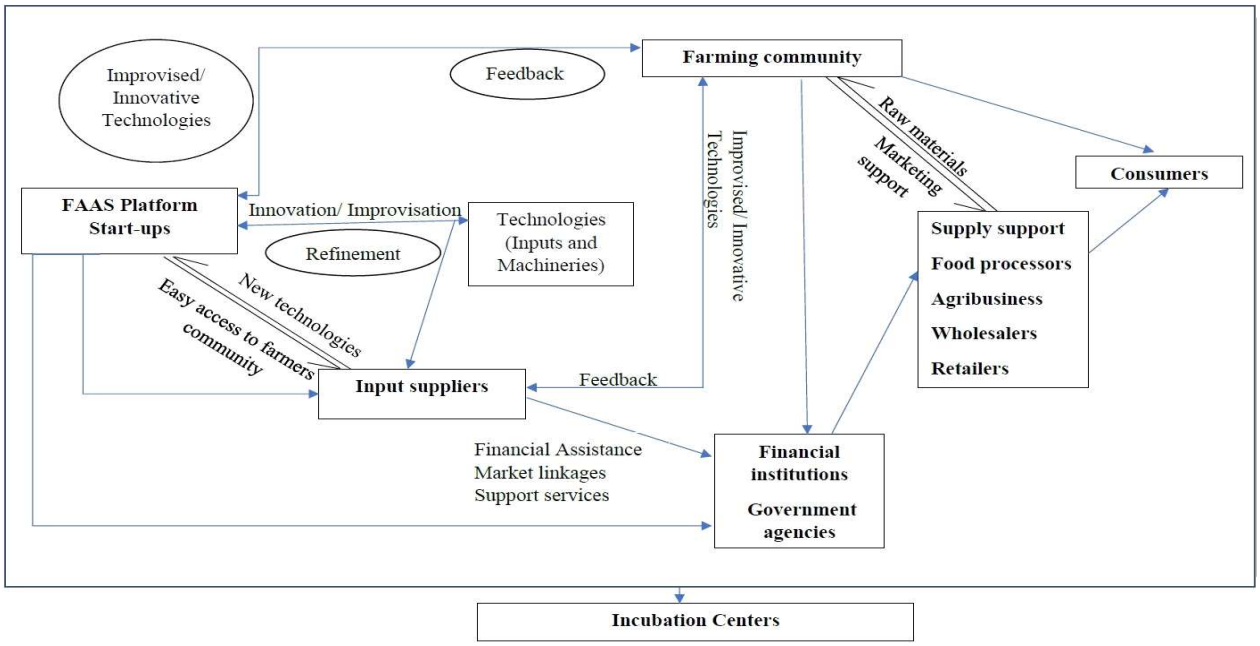

Fig. 1 : Farming As A Service (FAAS) Model

Stakeholders in the FAAS Ecosystem

Farmers are the Primary beneficiaries who access technology, inputs, knowledge, markets and financial services through the Start-up platform. Agritech Start-ups are Service providers bundling technology (IoT, AI, data analytics), advisory and market linkages into modular services for farmers. Input Suppliers are to provide seeds, fertilizers, equipment or services integrated into the platform for seamless farmer access. Financial Institutions/Fintech offer embedded credit, insurance and payment solutions to reduce farmer risk and improve cash flow. Agribusiness and Food Processors, as B2B clients, they can access raw materials and collaborate on quality standards. Government and Extension Services, support by supplying subsidies, policy incentives and extension support integrated into the platform. Consumers, ultimately benefit from better farm outputs, traceability and sustainability assurance via the platform's linkage with supply chains.

This integrated FAAS model leverages technology, finance and partnerships to create a scalable, sustainable pathway evolving startup business models beyond traditional sales to a service-centric ecosystem that empowers farmers and connects consumers.

As Start-ups do not have direct access to farming community many times, they can tie up with different extension units (Input suppliers, FPOs, RSKs etc) to make their innovation to targeted community. Farming community gives feedback about the technology to make the refinements. Then financial and government institutions aid all the stakeholders. This can be done through the Incubation centers which are playing a central role in Start-ups Ecosystem.

Conclusion and Recommendations

The results indicate the need for strengthening early-stage support (seed grants, prototype funding, pre-incubation) to improve survival rates of young Start-ups, promote rural incubation centers while extending the infrastructure support to semi-urban and rural areas. The predominance of scaling stage Start-ups signifies the need for growth-focused interventions along with the basic Start-up assistance. The sectoral differences (Agritech in Dharwad & Bengaluru; food processing in Mysuru) reflect the need for region-specific market opportunities. There is a need to encourage product diversification by offering R & D grants, product design labs and prototyping support to advisory-heavy start-ups along with imparting training to the stakeholders of ecosystem to give out effective advisory services to the target population. Government grants and bootstrapping were dominating as funding sources indicating dependence on public support and personal funds, with lower penetration of private investments. There is a need to introduce growth-stage financing schemes such as soft loans and revenue-based financing. Also, it is necessary to create awareness among the Start-ups about the different sources of funding such as angel investors and venture capitalists.

Outsourcing of technical functions and insourcing of product development and core R & D reflect strategic resource management but call for targeted support in digital, legal and compliance services. Limited benefits from ecosystem events, especially in Dharwad and Mysuru, imply the need for better-designed, region-specific networking, market access and capacity-building programmes. Scaling-up challenges related to funding, customer acquisition, talent building and risk from climate, market volatility and workforce highlight the need for financial facilitation, risk mitigation tools, supply-chain strengthening and rural-urban market expansion strategies. Overall, enabling policies should improve access to capital, strengthen technological and logistical infrastructure, promote innovation-led growth and ensure deeper ecosystem engagement to enhance Start-up performance across regions.

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Isolation and Screening of Indigenous Plant Growth-Promoting Rhizobacteria from Tomato (*Solanum lycopersicum* L) Rhizosphere Soils of Beggli Micro-Watershed, Kolar, Karnataka

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Abstract

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops cultivated worldwide; however, its productivity is greatly influenced by nutrient availability in the soil. Excessive chemical fertilizer usage has led to adverse effects on soil health and the environment, thereby necessitating the exploration of eco-friendly alternatives. Given the above, the present study was undertaken to isolate and characterize plant growth-promoting rhizobacteria (PGPR), including nitrogen-fixing bacteria (TNFB), phosphate-solubilizing bacteria (TPSB) and potassium-solubilizing bacteria (TKSB), from tomato rhizosphere soils of the Beggli micro-watershed, Kolar district, Karnataka. A total of 75 bacterial isolates were obtained using standard enrichment and serial dilution techniques. Based on in vitro screening, isolates TNFB-20, TPSB-13 and TKSB-17 were identified as the most efficient PGPR strains, exhibiting significantly higher nutrient solubilization efficiency and enhanced production of indole-3-acetic acid (IAA) and gibberellic acid (GA3). These findings highlight the potential of these native isolates as eco-friendly bioinoculants for improving tomato growth, yield and soil fertility, while reducing dependency on chemical fertilizers.

Keywords : Tomato rhizosphere, plant growth-promoting rhizobacteria, IAA, GA^3, Biofertilizer

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops cultivated worldwide and occupies a significant position in the Indian agricultural sector. India ranks second in global tomato production, with Karnataka being a major contributor due to its favorable agro-climatic conditions (FAO, 2021). Tomato requires high amounts of essential macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) for optimal growth, yield and fruit quality. However, the indiscriminate and excessive application of chemical fertilizers has led to environmental degradation, soil nutrient imbalances, and increased production costs (Sharma *et al*., 2023). The rhizosphere - the narrow region of soil directly influenced by root exudates - hosts diverse microbial populations that can significantly influence plant nutrition and health. Among them, plant growth-promoting rhizobacteria (PGPR) capable of fixing atmospheric nitrogen, solubilizing insoluble phosphate and mobilizing potassium have gained attention as sustainable alternatives to chemical fertilizers (Vessey, 2003 and Zaidi *et al*., 2015). These beneficial microbes not only improve nutrient uptake but also enhance root development, stress tolerance and disease resistance (Bhattacharyya and Jha, 2012).

Nitrogen-fixing bacteria, including *Azotobacter*, *Azospirillum* and certain *Bacillus* spp., convert atmospheric N, into plant-available forms (Ahmad *et al*., 2008). Phosphate-solubilizing bacteria (PSB), such as *Pseudomonas*, *Bacillus* and *Rhizobium*, release organic acids that chelate calcium and iron-bound phosphates, enhancing phosphorus availability (Rodrķguez and Fraga, 1999). Like wise, potassium-solubilizing bacteria (KSB) like *Bacillus mucilaginosus* and *Frateuria aurantia* play a critical role in releasing potassium from silicate minerals (Meena *et al*., 2015).

Watersheds serve as key hydrological units for managing land, water and soil resources in an integrated manner. They provide a functional landscape for implementing sustainable agricultural practices, especially in rainfed regions like the Kolar district. The Beggli micro-watershed represents a micro-level planning unit within the semi-arid region of Karnataka, where tomato is widely cultivated under intensive fertilizer regimes. Understanding the native microbial communities in agroecosystems is vital for developing eco-friendly and location-specific nutrient management strategies. Watershed-based studies also offer the advantage of linking soil biological health with spatial planning and sustainable land use. Hence, the present study was conducted to isolate and screening of nitrogen-fixing, phosphate-solubilizing and potassium-solubilizing bacteria from the rhizosphere soils of tomato crops in the Beggli micro-watershed, Kolar district, to identify efficient native strains for potential biofertilizer development.

Material and Methods

Collection of Soil Samples from Tomato Rhizosphere

Soil samples were collected from the Beggli micro-watershed, Kolar district, Karnataka, which lies in the Eastern Dry Zone of the state. The area is characterized by red sandy loam soils and moderate rainfall, making it ideal for tomato (*Solanum lycopersicum*) cultivation. Rhizospheric soil samples were collected from healthy tomato plants at four different locations within the Beggli micro-watershed. Samples were taken from a depth of 0-15 cm using a sterile spatula and transferred into sterile polythene bags. Each sample was labeled and transported to the laboratory under aseptic conditions. Samples were stored at 4 °C until further processing.

Isolation of Tomato Rhizobacteria

Isolation of rhizobacteria was performed using the serial dilution and spread plate technique. One gram of each soil sample was suspended in 9 ml of sterile distilled water and serially diluted up to 10^{-6}. From appropriate dilutions, 0.1 ml aliquots were plated onto different selective media: Jensen's Agar for free-living nitrogen-fixing bacteria. Pikovskaya's Agar for phosphate-solubilizing bacteria and aleksandros Agar for potassium-solubilizing bacteria. Nutrient Agar (NA) was used for maintenance of pure cultures. The plates were incubated at 28 ± 2 °C for 48-72 hours. Distinct colonies were selected based on morphology, purified by the four-way streak plate method and maintained on respective agar slants at 4 °C for further studies.

Screening of Bacterial Isolates under *In-vitro* Conditions

The bacterial isolates obtained were qualitatively and quantitatively screened for their nitrogen-fixing ability and phosphate- and potassium-solubilizing potential.

In-vitro Nitrogen Fixation

The nitrogen-fixing potential of bacterial isolates was assessed by using Jensen's agar medium at a dilution of 10^{-5}, which lacks combined nitrogen sources. Growth of colonies on this medium indicated the ability to fix atmospheric nitrogen. The nitrogen fixation by each bacterial isolate was estimated according to the method described by Humphries (1956). The Norris Glucose Nitrogen-Free Medium was used in this study. A 100 ml quantity of the above-mentioned medium was dispensed to a 250 ml capacity conical flask and autoclaved. The bacterial

cultures grown for 24 hrs separately in NFB broth were used to inoculate at 2 ml/100 ml of the medium. Triplicate samples were maintained for each isolate.

After seven days of incubation at 37 °C, the culture was homogenized. Five ml of the homogenized culture were collected and digested with 5 ml of concentration H_2SO_4 and 200 mg catalytic mixture K_2SO_4: $CuSO_4$: selenium (100:10:1 ratio) till the contents became clear. After cooling, the volume was made up to 25 ml with distilled water. Then aliquot of 5 ml was transferred to the micro-kjeldhal distillation unit. An aliquot of 10 ml of 40 per cent sodium hydroxide was added and steam distilled. Ammonia evolved was collected over 2 per cent boric acid (20 ml) containing 2 drops of double indicator (83.3 mg bromocresol green + 16.6 mg methyl red indicator dissolved in 10 ml of 95% ethanol) and back titrated against 0.005 N H_2SO_4. Percent N was calculated with following formula,

$$\text{Percent N} = \frac{\text{Titre value x 0.0014 x N of } H_2SO_4 \text{ x vol.made}}{\text{Volume of sample speed}} \times 100$$

Quantitative estimation of ammonia production was performed by inoculating the isolates into Jensen's broth and incubating at 28 ± 2 °C for 48 hours. After incubation, 1 ml of culture supernatant was mixed with Nessler's reagent and the development of a brownish-yellow color confirmed ammonia production. The concentration of ammonia was measured spectrophotometrically at 450 nm and expressed as mg NH ml^{-1}.

Phosphorus Solubilization Assay

Phosphate-solubilizing activity of microbial cultures was determined on Pikovskaya agar (Pikovskaya, 1948) containing 0.5 per cent (w/v) tricalcium phosphate. A clear zone's occurrence was considered a positive indication of phosphate solubilization (Nautiyal, 1999).

Quantitative analysis of tricalcium phosphate solubilization in a liquid medium was carried out using the Fiske and Subbarow (1925). Microbial isolates were inoculated into 50 ml of Pikovskaya's broth and incubated on a shaker at 120 rpm for 7 days at 28 ± 0.2 ^{0}C. The supernatant was collected by centrifugation at 12,000 rpm for 15 min. The resultant supernatant (500 µl) was mixed with 500 µl of 10 per cent (w/v) trichloroacetic acid in a test tube. Four milliliters of the color reagent (1:1:1:2 ratio of 3M H_2SO_4, 2.5 per cent ammonium molybdate, 10 per cent ascorbic acid and sterile distilled water) was added and the mixture was incubated at room temperature for 15 min. The pH value of the culture broth was measured on day 0 and day 7. Sterile PVK medium was served as a control. The optical density was read at 820 nm using a spectrophotometer. Soluble phosphorus was estimated using a standard curve.

The solubilizing index and efficiency of the microbes was calculated using the following formula (Pikovskaya, 1948).

Solubilizing efficiency (SE%) = $Z/C \times 100$

Solubilizing index (SI) = (C + Z)/× 100

Where, Z and C stand for clearance zone (mm) and colony diameter (mm), respectively.

Potassium Solubilization Assay

The potassium solubilizing activity of microbial cultures was determined on the Aleksandrow medium. Potassium aluminum silicate is usually used as a source of the insoluble form of K potassium (Meena *et al.,* 2016). The plates were then incubated at 30 °C. After 7 days, the colonies exhibiting clear zones were selected and the diameter of the solubilization zone was calculated in mm. Quantitative estimation of K solubilization was performed by flame photometry, wherein culture broth was centrifuged and the supernatant was used for precipitation of cobalt nitrite. The standard curve for the quantification of K is prepared using various concentrations of KCl solution (Hu *et al*., 2006).

Assessment of Plant Growth-Promoting Traits

Indole-3-Acetic Acid (IAA) production

The production of plant growth-promoting hormones, Indole-3-Acetic Acid (IAA) and Gibberellic Acid (GA), was quantitatively estimated for the nitrogen-fixing (TNFB), phosphate-solubilizing (TPSB) and potassium-solubilizing (TKSB) bacterial isolates.

Quantitative Estimation of IAA (Gordon and Paleg, 1957)

Bacterial isolates were grown in King's B medium supplemented with tryptophan (500 mg/L) and incubated at 28 ^{0}C for five days. The bacterial suspension was centrifuged at 7000 rpm for 5 min and the supernatant was collected. To the 2ml of supernatant 4ml of Salkowski reagent was added and allowed to stand at room temperature for 30 minutes. The appearance of pink color served as a positive result; optical density was measured by spectrophotometer at 535 nm and the amount of IAA was obtained by standard curve. The intensity of the pink color was read at 535 nm spectrophotometrically (Thermo Scientific, Biomate 3S, China) and the amount of IAA produced was calculated using standard curve.

Gibberellic Acid (GA) Production

Twenty-five ml of the fresh culture supernatant was taken in a test tube to which two ml of Zinc acetate was added. After two minutes, two ml of potassium ferrocyanide was added and centrifuged at 1000 rpm for 15 minutes. To five ml of this supernatant, five ml of 30 per cent hydrochloric acid was added and incubated at 20 ^{0}C for 75 minutes. The blank was maintained without cultures; similarly, the standard curve was prepared with known concentrations (100, 10, 1, 0.1 and 0.01 ppm) of GA. The absorbance was measured at 254 nm in a UV-visible spectrophotometer (Paleg 1965). The amount of GA present in the extract was calculated from the standard curve and expressed as µg/ml of the medium.

Statistical Analysis

All experiments were conducted in triplicate and the data were subjected to analysis of variance (ANOVA) using SPSS or WASP software. Significance was tested at $p < 0.05$ and results were expressed as mean ± standard error (Gomez and Gomez, 1984).

Results and Discussion

Plant growth-promoting rhizobacteria (PGPR) play a vital role in improving crop productivity by enhancing nutrient availability and stimulating plant growth through various direct and indirect mechanisms. In the present study, an attempt was made to isolate and screen efficient nitrogen-fixing, phosphate-solubilizing and potassium-solubilizing bacteria (NPK bacteria) from the tomato rhizosphere soils collected from the Beggli micro-watershed, Kolar district (Table 1). Four rhizosphere soil samples (TSB1, TSB2, TSB3 and TSB4) were analyzed to understand their nutrient status, which served as a basis for isolating efficient NPK-solubilizing PGPR (Plate 1). The diversity and efficiency of isolated strains were further assessed to identify potential candidates for improving tomato growth and soil fertility.

Physicochemical properties of the soil were analyzed and presented in Table 2. The soils from the Beggli micro-watershed were slightly acidic to neutral (pH 5.94-7.22), non-saline (ECE 0.35-0.52 dS/m) and exhibited medium to high fertility. Organic carbon ranged from 0.63-0.98%, with available N (170.52-211.00 kg/ha), P (27.43-41.36 kg/ha) and K (261.67-310.38 kg/ha), indicating favorable conditions for microbial activity and tomato growth.

Nitrogen Fixation Potential of Free-Living Microbial Isolates

The *In-vitro* nitrogen fixation ability of 25 microbial isolates from tomato rhizosphere soils of the Beggli micro-watershed varied significantly, ranging from 0.052% (TNFB-22) to 1.052% (TNFB-12, TNFB-20) (Table 3). Among all, TNFB-12 and TNFB-20 recorded the highest nitrogen

TABLE 1

Bacterial isolates from tomato rhizosphere soil samples of Beggli micro-watershed

Sample Code	Isolate Code	Isolate Description	Number of isolates
TSB (Tomato soil Beggli)	TNFB	Tomato Nitrogen-Fixer Beggli	25
	TPSB	Tomato Phosphate-Solubilizer Beggli	25
	TKSB	Tomato Potash-Solubilizer Beggli	25

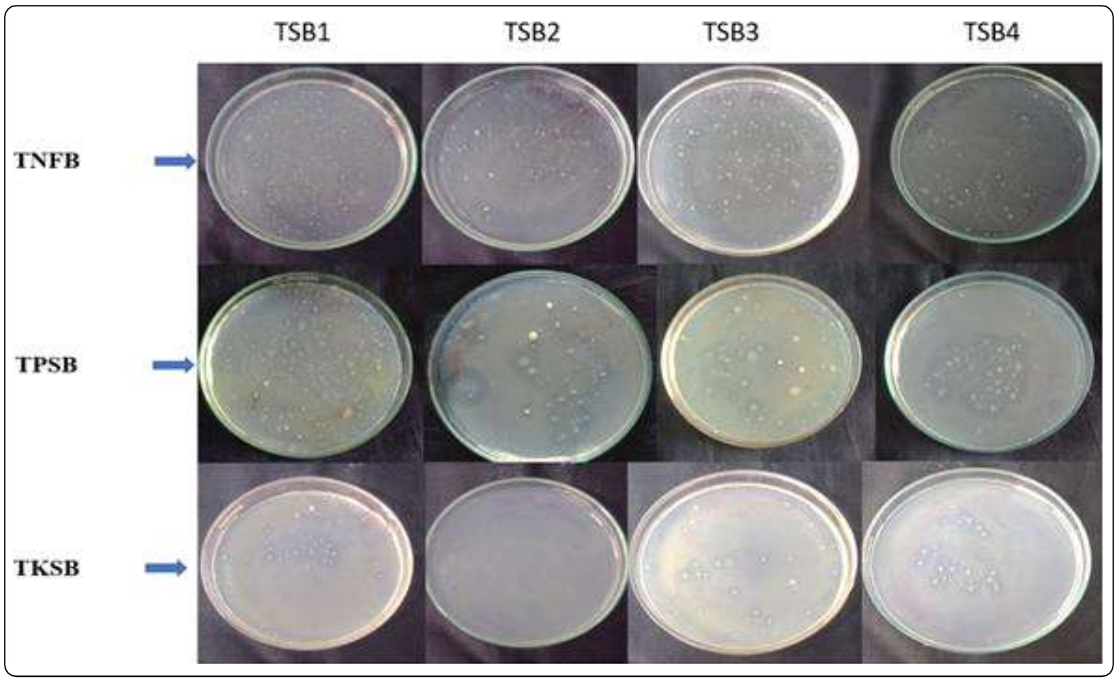


Plate 1 : Isolation of nitrogen-fixing (TNFB), phosphate-solubilizing (TPSB), and potassium-solubilizing (TKSB) bacterial isolates from tomato rhizosphere soils of Beggli micro-watershed at 10{ u dilution from four tomato rhizosphere soil samples (YTSB1. TSB2, TSB3, TSB4)

TABLE 2

Physicochemical properties of soil collected from tomato rhizosphere soils of Beggli micro-watershed

Physicochemical Properties	TSB1	TSB2	TSB3	TSB4
pH	6.78 ± 0.02	7.22 ± 0.01	6.60 ± 0.08	5.94 ± 0.02
ECE (dS/m)	0.45 ± 0.01	0.52 ± 0.00	0.48 ± 0.00	0.35 ± 0.02
OC (%)	0.78 ± 0.00	0.98 ± 0.02	0.85 ± 0.01	0.63 ± 0.00
N (kg/ha)	190.73 ± 0.64	211.00 ± 0.58	201.00 ± 0.58	170.52 ± 0.29
P (kg/ha)	32.00 ± 0.29	41.36 ± 0.32	35.39 ± 0.20	27.43 ± 0.23
K (kg/ha)	281.00 ± 3.79	310.38 ± 0.20	290.13 ± 0.07	261.67 ± 1.20

Note : All the values are mean ± standard error (n=3) at p d"0.05. TSB (Tomato soil Beggli), electrical conductivity (ECE), organic carbon (OC), available nitrogen (N), available phosphorus (P), available potassium (K)

fixation (1.052%), followed by TNFB-19 (0.942%) and TNFB-9, TNFB-11, TNFB-15, TNFB-23 (0.882%) (Table 3). The variation in nitrogen fixation efficiency could be attributed to differences in the metabolic potential and enzymatic activity of the isolates, particularly nitrogenase enzyme production, which plays a vital role in converting atmospheric nitrogen into plant-available forms.

These findings agree with earlier studies, which reported that *Azotobacter*, *Azospirillum* and other free-living diazotrophs exhibit varying nitrogen fixation capacities depending on soil properties, root exudates and microbial diversity (Rana *et al*., 2020 and Singh *et al*., 2021). Higher nitrogen-fixing isolates could play a significant role in enhancing soil fertility and reducing dependency on synthetic nitrogen fertilizers.

TABLE 3

***in-vitro* Nitrogen fixation by microbial isolates**

Microbial isolates	Percent N_2 fixation
TNFB-1	0.782
TNFB-2	0.552
TNFB-3	0.442
TNFB-4	0.382
TNFB-5	0.722
TNFB-6	0.492
TNFB-7	0.832
TNFB-8	0.612
TNFB-9	0.882
TNFB-10	0.782
TNFB-11	0.882
TNFB-12	1.052
TNFB-13	0.162
TNFB-14	0.272
TNFB-15	0.882
TNFB-16	0.872
TNFB-17	0.722
TNFB-18	0.872
TNFB-19	0.942
TNFB-20	1.052
TNFB-21	0.782
TNFB-22	0.052
TNFB-23	0.882
TNFB-24	0.872
TNFB-25	0.722
S. Em (±)	0.004
C.D. (P=0.01)	0.013

Ammonia Production by Free-Living Nitrogen-Fixing Isolate

Among the 25 free-living nitrogen-fixing isolates from tomato rhizosphere soils, several showed varying levels of ammonia production (Plate 2). Isolates TNFB-12, TNFB-20, TNFB-19 and TNFB-9 exhibited high ammonia production, while isolates like TNFB-3, TNFB-4 and TNFB-22 showed lower activity. These variations indicate differences in ammonification potential and nitrogenase enzyme activity, which play a vital role in enhancing soil nitrogen availability and promoting plant growth (Rana *et al*., 2020 and Singh *et al*., 2021).

Qualitative and Quantitative Screening of Bacterial Isolates for Phosphate-Solubilizing Ability

The phosphate solubilization efficiency of the 25 TPSB isolates varied significantly (Table 4). Among them, TPSB-29 exhibited the highest solubilizing efficiency (178.91%) with a solubilizing index (SI) of 3.27, followed by TPSB-18 (167.04%, SI = 2.65), TPSB-26 (161.87%, SI = 3.01), TPSB-12 (152.74%, SI = 2.66) and TPSB-15 (146.94%, SI = 3.05). Conversely, isolates such as TPSB-4 and TPSB-7 showed relatively lower efficiencies (90.34% and 11.54%, respectively). A significant decrease in pH was observed in efficient isolates (from 7.0 in control to as low as 5.40 in TPSB-29), correlating with higher soluble phosphorus (Pi) release (3.84 µg/ml in TPSB-29). The trend indicates that increased organic acid production by efficient phosphate-solubilizing bacteria enhances P solubilization, leading to a decrease in broth pH and higher Pi release. Phosphate solubilizing bacteria (PSB) enhance phosphorus availability by secreting

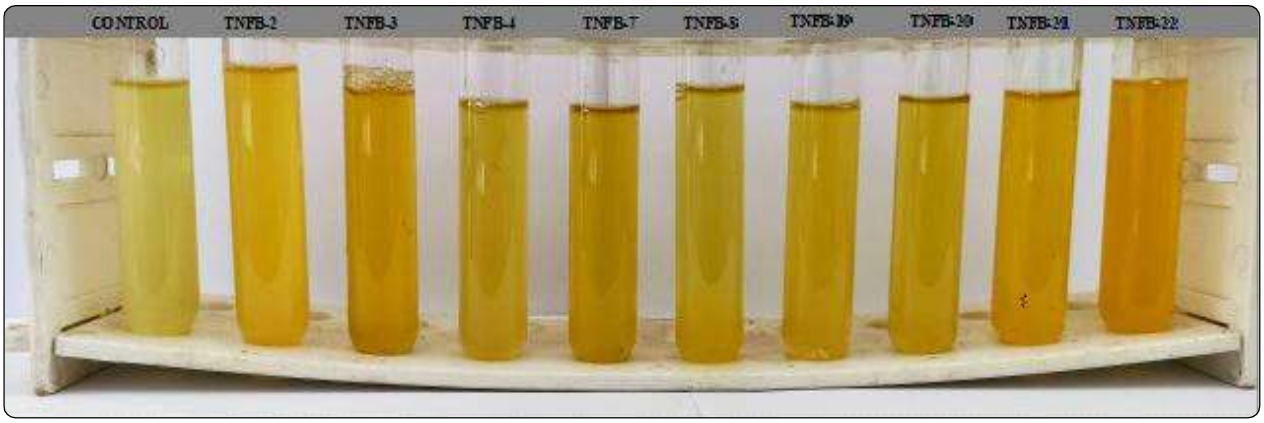


Plate 2 : Ammonia production by free- living nitrogen fixing isolates of tomato rhizosphere soils samples

TABLE 4

Release of Pi from TCP broth by microbial isolates after seven days of incubation

Bacterial isolates	7 Days after incubation (DAI)			
	pH	Pi (µg/ml)	Solubilizing index (SI)	Solubilizing efficiency (SE %)
TPSB-1	5.66	0.93	-	-
TPSB-2	5.71	0.93	-	-
TPSB-3	5.56	1.06	2.09	111.54
TPSB-4	6.01	0.92	1.98	90.34
TPSB-5	6.91	0.69	-	-
TPSB-6	6.00	0.92	-	-
TPSB-7	5.51	1.12	2.09	11.54
TPSB-8	5.51	1.12	-	-
TPSB-9	5.51	1.12	-	-
TPSB-10	5.50	1.13	2.28	120.34
TPSB-11	5.51	1.93	2.18	122.54
TPSB-12	5.41	3.27	2.66	152.74
TPSB-13	5.51	2.73	2.28	122.64
TPSB-14	5.50	3.13	2.90	142.94
TPSB-15	5.41	3.27	3.05	146.94
TPSB-16	5.51	3.13	2.48	140.34
TPSB-17	5.71	0.93	-	-
TPSB-18	5.40	3.8	2.65	167.04
TPSB-19	6.90	0.68	-	-
TPSB-20	6.80	0.83	-	-
TPSB-21	6.91	0.72	-	-
TPSB-22	7.01	0.64	-	-

Continued....

Table 1 Continued....

Bacterial isolates	7 Days after incubation (DAI)			
	pH	Pi (µg/ml)	Solubilizing index (SI)	Solubilizing efficiency (SE %)
TPSB-23	6.91	0.82	-	-
TPSB-24	5.41	3.60	3.01	161.87
TPSB-25	5.40	3.84	3.27	178.91
Control	7.0	0.63	-	-
S. Em (±)	0.007	0.070	0.044	0.434
C.D. (P=0.01)	0.019	0.199	0.128	1.265

organic acids, which chelate cations and reduce pH, thereby increasing Pi release (Kumar and Naik 2021). Similar findings were reported by Khan *et al* (2009), where highly efficient PSB isolates exhibited a strong correlation between pH reduction and phosphate solubilization.

Qualitative and Quantitative Screening of Bacterial Isolates for Potassium Solubilizing Ability

potassium solubilization efficiency of the 25 TKSB isolates varied widely (Table 5). Among them, TKSB-17 exhibited the highest solubilizing efficiency (331.14%) with a solubilizing index (SI) of 2.31, followed by TKSB-1 (269.77%, SI = 2.26), TKSB-24 (157.24%, SI = 0.91), TKSB-15 (182.19%, SI = 1.31) and TKSB-10 (119.97%, SI = 2.24). On the other hand, isolates such as TKSB-2 (89.94%) and TKSB-4 (108.48%) showed relatively lower solubilization efficiencies. A significant decrease in pH was recorded in highly efficient isolates (5.40-5.80), which corresponded with enhanced K release in the broth. For instance, TKSB-17 released the highest potassium (4.88 µg/ml) with a substantial pH drop, indicating strong production of organic acids responsible for mineral solubilization.

The increased K solubilization by efficient isolates is attributed to the secretion of organic and inorganic

Table 5

***in vitro* pH and K release by microbial isolates in Aleksandrow broth**

Microbial isolates	7 Days after incubation (DAI)			
	pH	K (µg/ml)	Solubilizing index (SI)	Solubilizing efficiency (SE %)
TKSB-1	6.60	0.05	2.26	269.77
TKSB-2	5.80	2.49	1.35	89.94
TKSB-3	5.70	0.02	-	-
TKSB-4	6.10	2.44	1.21	108.48
TKSB-5	7.00	5.88	-	-
TKSB-6	6.10	3.58	1.31	129.94
TKSB-7	5.80	3.27	-	-
TKSB-8	5.50	2.86	-	-

Continued....

TABLE 5 Continued....

Microbial isolates	7 Days after incubation (DAI)			
	pH	K (µg/ml)	Solubilizing index (SI)	Solubilizing efficiency (SE %)
TKSB-9	5.60	1.92	-	-
TKSB-10	5.40	2.87	2.24	119.97
TKSB-11	5.50	9.61	-	-
TKSB-12	5.50	10.09	-	-
TKSB-13	5.60	7.14	-	-
TKSB-14	5.80	0.92	1.26	124.94
TKSB-15	5.40	2.64	1.31	182.19
TKSB-16	5.60	3.13	-	-
TKSB-17	5.80	4.88	2.31	331.14
TKSB-18	5.40	4.60	-	-
TKSB-19	7.00	0.92	-	-
TKSB-20	6.90	4.88	-	-
TKSB-21	6.50	3.14	-	-
TKSB-22	6.60	2.86	-	-
TKSB-23	7.00	14.90	-	-
TKSB-24	7.00	4.88	0.91	157.24
TKSB-25	6.90	1.18	-	-
Control	7.00	0.01	-	-
S. Em (±)	0.058	0.006	0.021	0.174
C.D. (P=0.01)	0.164	0.016	0.062	0.518

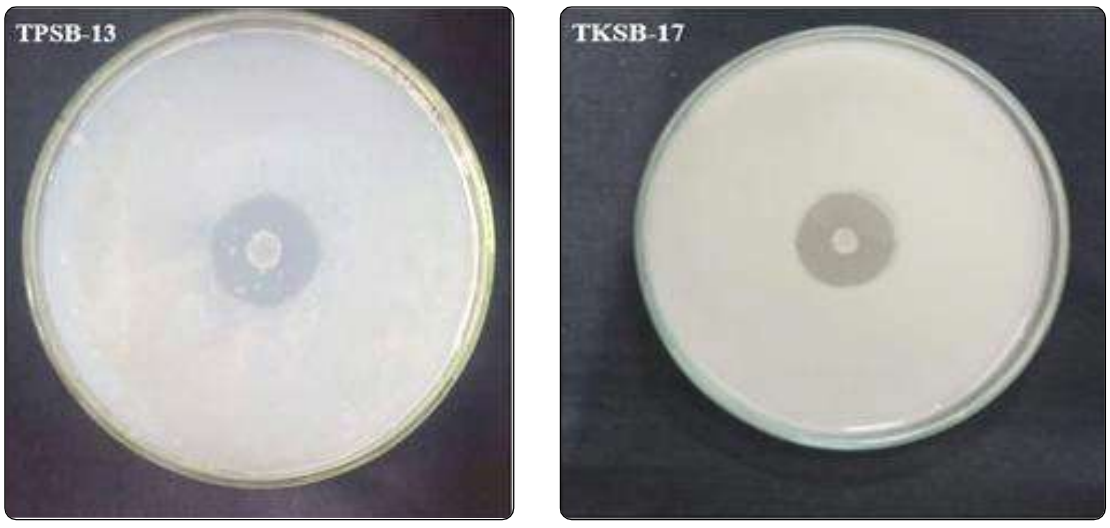


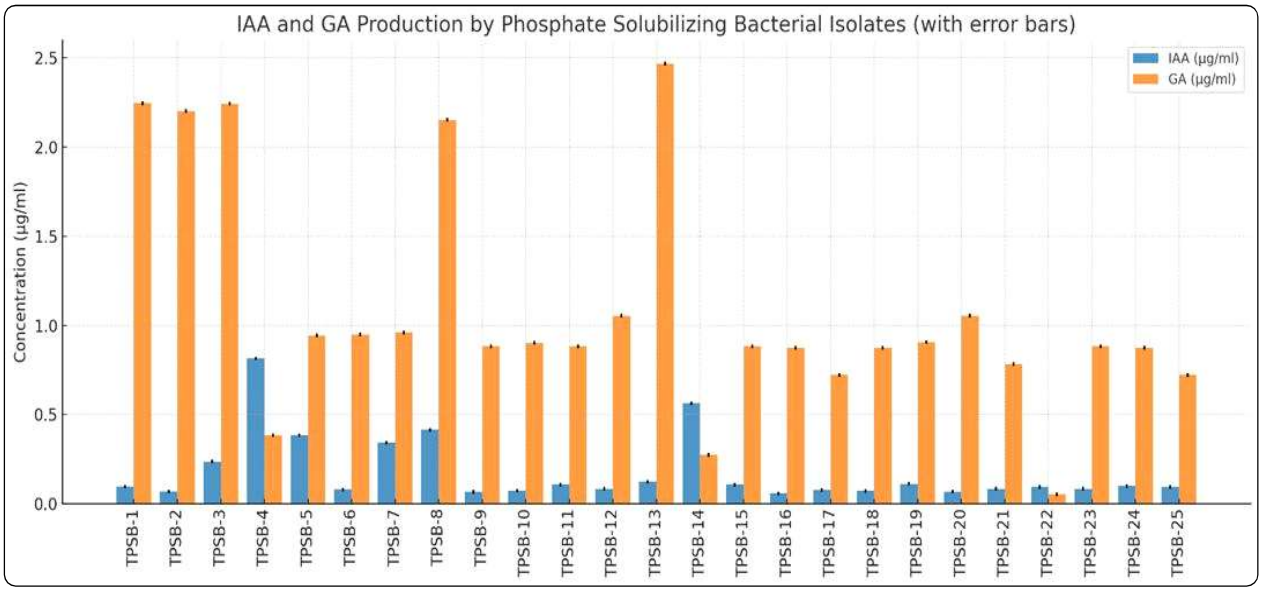
Plate 3 : Bacterial isolates showing clear zone formation indicating phosphate (TPSB-13) and potassium (TKSB-17) solubilization

acids, which lower the pH and facilitate the dissolution of insoluble potassium-bearing minerals. Similar findings were reported by Archana *et al*. (2013), where higher organic acid production was strongly correlated with enhanced K release from mica and feldspar.

IAA and GA Production by Free-living N, -fixers, Phosphate and Potash Solubilizing Isolates

The production of indole-3-acetic acid (IAA) and gibberellic acid (GA) varied significantly among the nitrogen-fixing (TNFB), phosphate-solubilizing (TPSB) and potash-solubilizing (TKSB) microbial isolates, highlighting the strain-specific capacity to synthesize plant growth-promoting hormones (Fig. 1). Among the free-living nitrogen-fixing isolates, TNFB-20 exhibited the highest IAA (1.41 µg/ml) and GA (1.911 µg/ml) production, followed by TNFB-16, TNFB-12 and TNFB-19 (Fig. 1A), indicating their strong potential to enhance root development, cell elongation and nutrient uptake (Patten & Glick, 2002). In contrast, phosphate-solubilizing isolates showed overall lower IAA production but a few strains like TPSB-13 (IAA = 0.123 µg/ml, GA = 2.467 µg/ml) and TPSB-1 (IAA = 0.096 µg/ml, GA = 2.248 µg/ml) demonstrated exceptionally high GA synthesis (Fig. 1B). Such isolates are particularly valuable in promoting stem elongation, flowering and seed germination, especially under P-deficient soils (Mehnaz & Lazarovits, 2006). Among the potash-solubilizing isolates, TKSB-1 (IAA = 1.89 µg/ml, GA = 2.161 µg/ml) and TKSB-10 (IAA = 1.548 µg/ml, GA = 2.474 µg/ml) were the most efficient producers, suggesting their dual role in potassium mobilization and phytohormone synthesis (Fig. 1C), thereby improving water regulation, enzyme activation and overall crop vigor (Parmar & Sindhu, 2013). Overall, isolates TNFB-20, TPSB-13 and TKSB-10 emerged as the most promising strains due to their combined capacity for nutrient solubilization and phytohormone production. Their utilization as biofertilizer candidates can reduce chemical fertilizer dependency while enhancing tomato growth and productivity sustainably (Murali *et al*., 2025).

This is the first preliminary study on the isolation and screening of indigenous plant growth-promoting rhizobacteria (PGPR) from the tomato rhizosphere soils of the Beggli micro-watershed, Kolar, Karnataka. The study successfully identified diverse potassium-solubilizing bacteria (TKSB), phosphate-solubilizing bacteria (TPSB) and total nitrogen-fixing bacteria (TNFB) with significant potential to enhance tomato growth and soil fertility. Among the isolates, TKSB-17, TPSB-13 and TNFB-20 emerged as the most efficient strains based on their higher nutrient



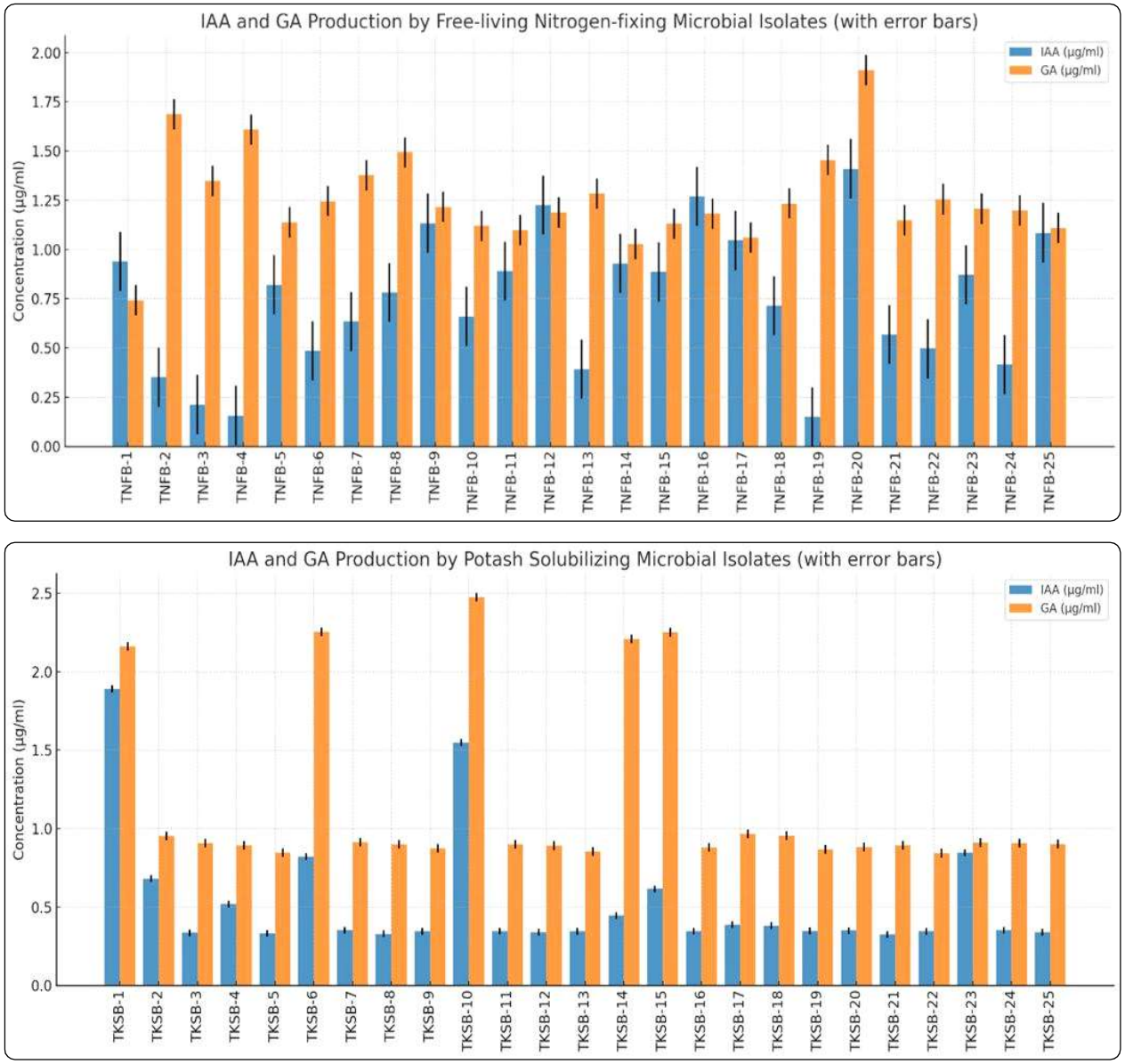


Fig. 1 : IAA and GA production by Bacterial isolates of tomato rhizosphere soil. A) TFNB, B) TPSB, C) TKSB

solubilization, nitrogen-fixation potential and production of phytohormones such as IAA and GA. These findings indicate that the native isolates from the tomato rhizosphere have considerable potential as biofertilizers for improving nutrient availability and promoting sustainable tomato production. However, further evaluation under greenhouse and field conditions is essential to confirm their efficacy and suitability as effective bio-inoculants for tomato cultivation in this region.

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Isolation and Characterization of Bacterial Leaf Spot in Tomato caused by *Xanthomonas euvesicatoria* pv. *euvesicatoria*

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Abstract

Tomato (*Solanum lycopersicum* L.) is a globally important vegetable crop, but its production is severely hampered by bacterial leaf spot (BLS), caused by a group of *Xanthomonas* species. In the present study, symptomatic leaf and fruit tissues were collected from tomato plants and subsequently four bacterial isolates were purified and subjected to morphological, biochemical and molecular characterisation. The colonies were yellow, round, mucoid and Gram-negative with positive catalase and KOH solubility reactions, consistent with *Xanthomonas* spp. The Molecular confirmation using 16S rRNA gene amplification and sequencing revealed e ≥98% identity with *Xanthomonas euvesicatoria*. Pathogenicity tests on the susceptible cultivar Shivam exhibited progressive disease development, with mean severity values of 15.77% (3 DPI), 28.53% (6 DPI), 39.73% (9 DPI) and 72.29% (12 DPI). The bacterial colonisation studies indicated a steady increase in population from 6.1 × 10v CFU/ml (3 DPI) to 1.88 × 10w CFU/ml (12 DPI). These findings confirm *X. euvesicatoria* as the causative pathogen of BLS in the region and the study demonstrated its aggressive colonisation and pathogenic potential in susceptible tomato genotypes. The study underscores the importance of monitoring pathogen populations to understand their virulence spectrum and epidemiological significance.

Keywords : Xanthomonas, Tomato, Biochemical test, Disease severity, Colonisation

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide, valued not only as a dietary staple rich in vitamins, minerals and antioxidants but also as a significant source of income for farmers and the agro-based industries (Prabhu *et al*., 2017). The global tomato production has steadily increased over the past decades; however, its sustainability is increasingly threatened by several biotic and abiotic stresses. Among these, foliar bacterial diseases pose a considerable challenge to both open-field and protected cultivation systems. In particular, Bacterial Leaf Spot (BLS), caused by members of the genus *Xanthomonas*, is considered one of the most devastating diseases of tomatoes (Apoorva *et al*., 2023). BLS is a disease complex involving four phylogenetically related bacterial species: *Xanthomonas euvesicatoria*, *X. perforans*, *X. vesicatoria* and *X. gardneri*. The disease manifests as small, water-soaked lesions that expand into necrotic spots with chlorotic halos on leaves, stems and fruits. Severe infections may result in extensive defoliation, exposing fruits to sunscald, while fruits themselves develop conspicuous spots and blemishes that drastically reduce their market value (Priyanka *et al*., 2022). In epidemic situations, yield losses of up to 60% have been reported, particularly under warm and humid conditions that favour bacterial multiplication and spread. The management of BLS remains a formidable challenge due to the biological and ecological features of the pathogen. *Xanthomonas* species are

primarily seedborne, enabling long-distance dissemination through commercial seed trade and complicating the production of pathogen-free seed lots. Furthermore, the pathogen exhibits high genetic diversity and remarkable adaptability (Singh and Jagadish, 2018). These attributes facilitate the rapid emergence of new, highly aggressive strains that can overcome host resistance genes. Consequently, resistant cultivars often lose effectiveness within a few growing seasons. The chemical control using copper-based bactericides has been the conventional practice for decades, but their long-term efficacy has been compromised by the widespread emergence of copper-tolerant strains. The Integrated management strategies combining crop hygiene, certified seeds and resistant varieties have only achieved partial success, thereby underscoring the need for continuous pathogen monitoring and novel resistance sources. The present study focuses on four isolates of *Xanthomonas euvesicatoria* (GXe1, GXe2, GXe3 and GXe4) collected from naturally infected tomato plants. The leaf and fruit samples showing typical BLS symptoms were processed and pure bacterial cultures were established. These isolates were subjected to pathogenicity assays on the susceptible tomato cultivar Shivam under controlled conditions. The disease progression was assessed through severity scoring and bacterial population dynamics, while biochemical and molecular approaches were employed for species confirmation and genetic characterization. The outcomes of this work provide insights into the pathogenic diversity of *X. euvesicatoria* isolates and highlight the necessity of systematic monitoring of pathogen populations for effective disease management and resistance breeding in tomato.

Material and Methods

Sample Collection and Isolation of *Xanthomonas*

Bacterial leaf spot infected leaf and fruit samples of tomato (*Solanum lycopersicum* L.) were collected from the University of Agricultural Sciences, GKVK campus, Bengaluru, Karnataka, India. The infected portions were surface sterilized with 70 per cent ethanol for 30 seconds, followed by three rinses with sterile distilled water to remove any residual disinfectant. The infected tissues were crushed and macerated in sterile distilled water. The resulting suspension was streaked onto nutrient agar (NA) plates, sucrose nutrient agar (SNA) and glucose yeast extract peptone agar(GYPA) and incubated at 28/ ±/ 2°C for 48 hours. The isolated individual colonies were selected and restreaked to obtain pure cultures for further analysis.

Pathogenicity Test

The susceptible Shivam cultivar was used for the pathogenicity assays under controlled glass house conditions (28 ± 2 °C, 85% relative humidity, 16 h light/8 h dark). At 32 days post sowing, single colonies of each bacterial isolate were cultured in nutrient broth for 48 h, then adjusted to $1x10^8$ CFU/ mL(OD_{600}=0.3). For syringe infiltration, this bacterial suspension was pressure injected into a 1-2 cm² area of the abaxial leaf midrib; each treatment included both biological and technical replicates arranged in a completely randomised design (CRD). After inoculation, plants were maintained at 28 ± 2 °C, 16 h photoperiod and 85 per cent RH. Disease severity was scored at 0, 3, 6, 9 and 12 days post-inoculation using the 0-5 scale of Vallejos *et al*. (2010).

Biochemical Characterisation of *Xanthomonas* Isolates

The colonies exhibiting characteristic yellow pigmentation and mucoid, presumed to be *Xanthomonas* spp., were selected for biochemical characterisation studies. The biochemical analysis of *Xanthomonas euvesicatoria* pv. *euvesicatoria* was carried by adapting the protocol of Fan *et al*., 2022. The Gram reaction and cellular morphology of the isolates were determined by Gram staining: smears of 24 hr culture were heat fixed on clean glass slides, stained with crystal violet (1 min), treated with Gram's iodine (1 min), decolorized in 95 per cent ethanol (30 s) and counterstained with

safranin (1 min), then examined under microscope. The Catalase activity was assessed by placing a loopful of fresh culture on a slide and adding 1-2 drops of 3 per son H_2O_2 observing for effervescence. The 3 per cent KOH test was performed by mixing a loopful of culture with a drop of KOH on a slide for 1 min and then observing for the formation of a viscous, mucoid thread upon lifting the loop. To assess oxidase activity, commercially available discs containing N, N, N2, N2, N2-tetramethyl-p-phenylenediamine were moistened with sterile distilled water and inoculated by smearing a 24 hr bacterial colony.

Molecular Characterization using 16S rRNA

The Genomic DNA was extracted from pure cultures of *Xanthomonas* isolates grown on nutrient broth at 28/ ±/ 2°C for 48 hr using a modified sucrose, Tris-EDTA buffer Method (Weisburg *et al*., 1991). A loopful of bacterial culture was suspended in 500/ µL of TE buffer, followed by the addition of 100/ µL of 10 per cent SDS and 20/ µL of Proteinase K (20/ mg/ mL). Subsequently, 100/ µL of CTAB/NaCl solution was added and the suspension was incubated at 65°C for 10 minutes. The DNA was extracted using an equal volume of chloroform: isoamyl alcohol (24:1) and the aqueous phase was precipitated with 600/ µL of ice cold isopropanol. The DNA pellet was washed with 70 per cent ethanol, air-dried and resuspended in 50/ µL of TE buffer. For 16S rRNA gene amplification, universal primers 27F (52 AGAGTTTG ATCMTGGC TCAG-32) and 1492R (52 TACGGYT ACCTTGTTACGACTT-32) were used in a 25/ µL PCR reaction containing 12.5/ µL of 2x PCR Master Mix, 1/ µL each of forward and reverse primers (10/ µM), 2/ µL of genomic DNA and 8.5/ µL of nuclease-free water (Csaikl *et al*., 2008). The PCR was carried out with initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 1 minute and extension at 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes. PCR products were confirmed by 1.2 per cent agarose gel electrophoresis and sequenced commercially. The sequences were analysed using BLASTn against the NCBI Gen Bank database for pathogen identification.

Assessment of Bacterial Density

The bacterial colonization density in infected leaf tissue was determined as follows. The symptomatic leaves (0.5 g) were surface sterilized, rinsed and homogenized in 10 mL of sterile 0.1 M phosphate buffer (pH 7.0) using an autoclaved mortar and pestle. The resulting suspension was serially diluted (10^{-1} to 10^{-6}) in the same buffer and 100 µL aliquots of each dilution were spread onto nutrient agar plates in triplicate. The plates were incubated at 28 °C for 48 h, after which colony forming units (CFU) were counted and expressed per gram of fresh tissue.

Histochemical Staining for Bacterial Colonization

For histochemical assessment of bacterial colonization, 2 mm × 3 mm leaf segments were excised from infection sites and transferred to a small Petri dish containing 10 µL each of propidium iodide (20 mM) and SYTO™ 9 nucleic acid stain (3.34 mM) from the LIVE/DEAD™ BacLight™ kit (Invitrogen) following the protocol of Boulos *et al*., 1999. The samples were incubated in the dark for 30 min, then washed three times with phosphate buffered saline to remove excess dye. The stained fragments were mounted in a drop of immersion oil on glass slides, covered with a coverslip and visualized under an EVOS M700 fluorescence microscope (Thermo Fisher Scientific), with propidium iodide indicating dead cells (red fluorescence) and SYTO 9 labelling live and dead bacteria (green fluorescence).

Statistical Analysis

Statistical analyses were conducted in R (Version 4.2.0; R Core Team, 2023). All experiments comprised three biological replicates and data were presented as mean ± standard error (SE). Pairwise comparisons of means were performed by Duncan's multiple range test (DMRT) at a significance level of $P < 0.05$. The bar graphs illustrating disease severity scores and bacterial colonization densities were generated with ggplot2, displaying error bars

for SE and letters denoting statistically homogeneous groups as determined by DMRT.

Results and Discussion

Isolation and Morphological Features of *Xanthomonas* Isolates

The bacterial isolates isolated from infected tomato leaves showing typical small, water soaked lesions with chlorotic halos produced characteristic colonies that were yellow, round, smooth, mucoid and convex. The colony features are indicative of *Xanthomonas* species, particularly due to the presence of the yellow xanthomonadin pigment (Fig. 1). The confirmed colonies were subsequently subjected to biochemical and molecular characterization for definitive identification. The symptomatology observed in the present study, small, water-soaked lesions with chlorotic halos, is consistent with earlier reports describing bacterial spot of tomato caused by *Xanthomonas* spp. (Jones *et al*., 2000; Ciardi *et al*., 2000 and Achari *et al*., 2019). The recovered colonies exhibited morphological traits typical of *Xanthomonas*, including yellow pigmentation due to xanthomonadin, a membrane-bound brominated aryl polyene pigment unique to this genus. Xanthomonadin is known to protect bacterial cells from photodamage and oxidative stress, contributing to pathogen survival on leaf surfaces (Poplawsky & Chun, 1997 and Abrahamian *et al*., 2021). Similar morphological characteristics of *X. euvesicatoria* isolates from tomato have been reported by Obradovic *et al*. (2004) and Timilsina *et al*. (2020), further supporting the identification of the pathogen in the present study.

Biochemical Properties of *Xanthomonas euvesicatoria*

The biochemical characterization of the isolates results is consistent with the typical profile of *Xanthomonas euvesicatoria*. Gram staining revealed slender, uniformly pink rods, indicative of Gram-negative morphology. The oxidase test was negative, as evidenced by the absence of purple coloration on oxidase discs within 30 seconds. In the 3 per cent KOH solubility test, cultures produced an immediate viscous, mucoid thread, further confirming the Gram-negative cell wall structure. Catalase activity was positive, with rapid effervescence observed upon exposure to 3 per cent H_2O_2, indicating the presence of catalase enzyme. The combined oxidase-negative, KOH-positive and catalase-positive reactions unequivocally supported the identification of the isolates as *X. euvesicatoria* (Fig. 2). The biochemical traits observed in this study align with established diagnostic profiles for *X. euvesicatoria* (Schaad *et al*., 2001 and Jones *et al*., 2004). The negative oxidase reaction and positive catalase activity are hallmark features of *Xanthomonas* spp., reflecting their aerobic metabolism and lack of cytochrome c oxidase. The immediate formation of a viscous thread in the KOH solubility test further corroborates the Gram-negative cell wall structure, as previously reported by Elliott and Stead (1987); Griffin *et al*., 2017. Similar biochemical profiles have been documented in *X. euvesicatoria* isolates from tomato

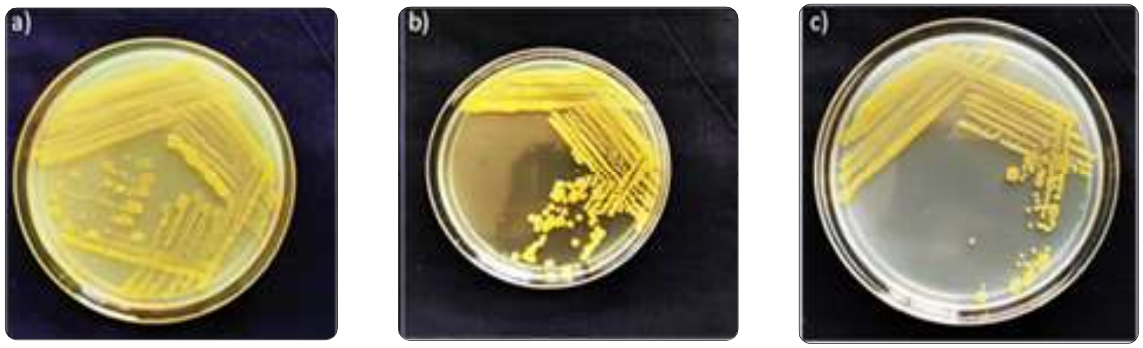

Fig. 1 : Cultural characterization of the GXe4. a) Sucrose Nutrient Agar b) Nutrient Agar c) Glucose Yeast extract Peptone Agar

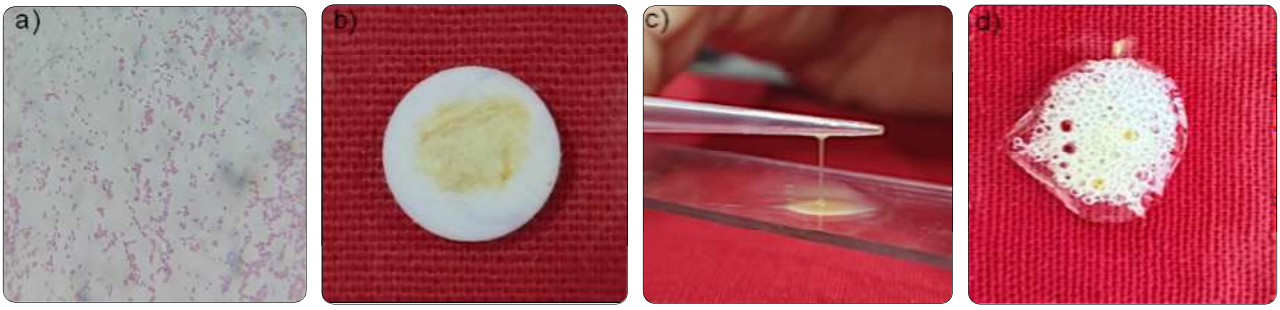

Fig. 2 : Biochemical characterization of the GXe4. a) Gram staining b) Oxidase test c) 3% KOH d) Catalase test

and pepper in diverse geographic regions (Jones *et al*., 1986; Obradovic *et al*., 2004; Timilsina *et al*., 2020), reinforcing the reliability of these assays for preliminary identification.

Molecular Confirmation of Isolates

The molecular confirmation of the isolates was achieved by amplifying the 16S rRNA gene using universal primers, which yielded ~1.5 kb products visualized on a 1.2 per cent agarose gel. The amplicons were sequenced and BLAST analysis revealed >99% identity with *Xanthomonas euvesicatoria* reference strains in the NCBI Gen Bank database. The 16S rRNA gene sequence of *Xanthomonas euvesicatoria* isolate Gxe4 was queried against the NCBI nucleotide database using the BLASTN algorithm. The best hit corresponded to *X. euvesicatoria* strain OQ151978.1 with >99% identity and full query coverage, supporting its assignment to this species. Additional close matches included other *X. euvesicatoria* strains such as LMG 27969 and NCPPB 4321, all showing high sequence similarity (>98%). The sequence for Gxe4 was subsequently submitted to GenBank under accession number [PX096578]. The Multiple sequence alignment of Gxe4 and selected *Xanthomonas* reference sequences was performed using MEGA11. A Maximum Likelihood tree was generated under the Kimura 2-parameter model, with 1,000 bootstrap replicates to test clade robustness. The resulting Fig. 3 shows isolate Gxe4 clustering tightly with *X. euvesicatoria* OQ151978.1 and other *X. euvesicatoria* strains, forming a strongly supported monophyletic clade

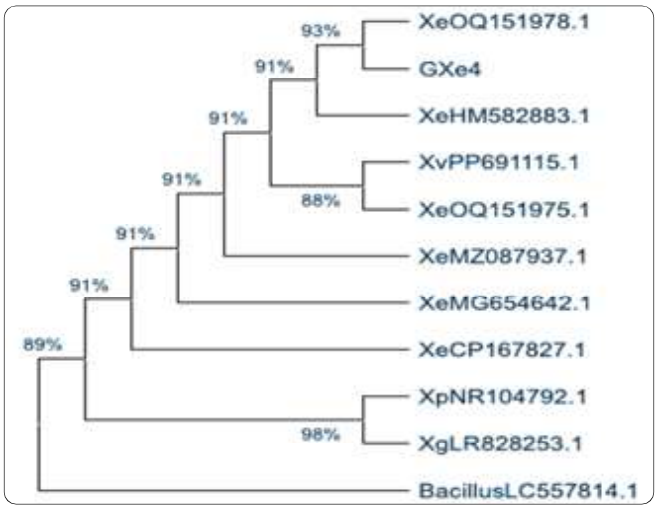

Fig. 3 : Molecular phylogenetic analysis of *Xanthomonas* species associated with bacterial spot using 16S rRNA gene sequences

(bootstrap support: 99%). This clade was clearly separated from other *Xanthomonas* species such as *X. perforans* and *X. gardneri*. *Bacillus* served as the outgroup to root the tree. The 16S rRNA gene is a well-established marker for bacterial taxonomy and identification due to its conserved and variable regions (Weisburg *et al*., 1991 and Ammons *et al*., 2002) and similar approaches for *X. euvesicatoria* have been reported in tomato and pepper (Obradovic *et al*., 2004 and Timilsina *et al*., 2020). The phylogeny also distinguished the bacterial spot complex into four lineages: *X. euvesicatoria* (Xe), *X. perforans* (Xp), *X. vesicatoria* (Xv) and *X. gardneri* (Xg). The isolate GXe4 clustered within the Xe clade, confirming its identity. Within the tree, *Xe* and *Xp* appeared as sister groups, reflecting recent divergence, while *Xv* was more distantly related and *Xg* formed an independent branch representing a distinct evolutionary trajectory. This phylogeny underscores the genetic distinctness of GXe4 and clarifies the evolutionary relationships among the four species responsible for bacterial spot in tomato.

Pathogenicity Assays

Disease severity in the susceptible tomato cultivar Shivam increased progressively with advancing days after inoculation (Fig. 4 & Table 1). At 3 DPI, the mean severity was 15.77%, which rose to 28.53% at 6 DPI and 39.73% at 9 DPI. The highest severity was recorded at 12 DPI, reaching 72.29%, indicating rapid disease development during later stages of infection. The steady rise in severity over time reflects the active colonization and expansion of *Xanthomonas euvesicatoria* within host tissues (Fig. 5). The sharp increase between 9 and 12 DPI corresponds

Fig. 4 : Pathogenicity assay on *Shivam* cultivar at (a) 3 dpi, (b) 6 dpi, (c) 9 dpi and (d) 12 dpi (dpi = days post inoculation)

Table 1

Disease severity, bacterial population and progression of *Xanthomonas euvesicatoria* in tomato cultivar Shivam at different days post inoculation (DPI)

Days Post Inoculation (DPI)	Disease Severity (%) (Mean ± SD)	Bacterial Population (CFU/ml) (Mean ± SD)
3 DPI	15.77 ± 0.30	6.10 × 10^6 ± 0.10
6 DPI	28.53 ± 0.27	9.67 × 10v ± 0.15
9 DPI	39.73 ± 0.28	1.38 × 10w ± 0.15
12 DPI	72.29 ± 0.38	1.88 × 10w ± 0.20
SE(m)	0.19	6.67 × 10t
CD (5%)	0.61	2.17 × 10u
CV%	0.83	0.95

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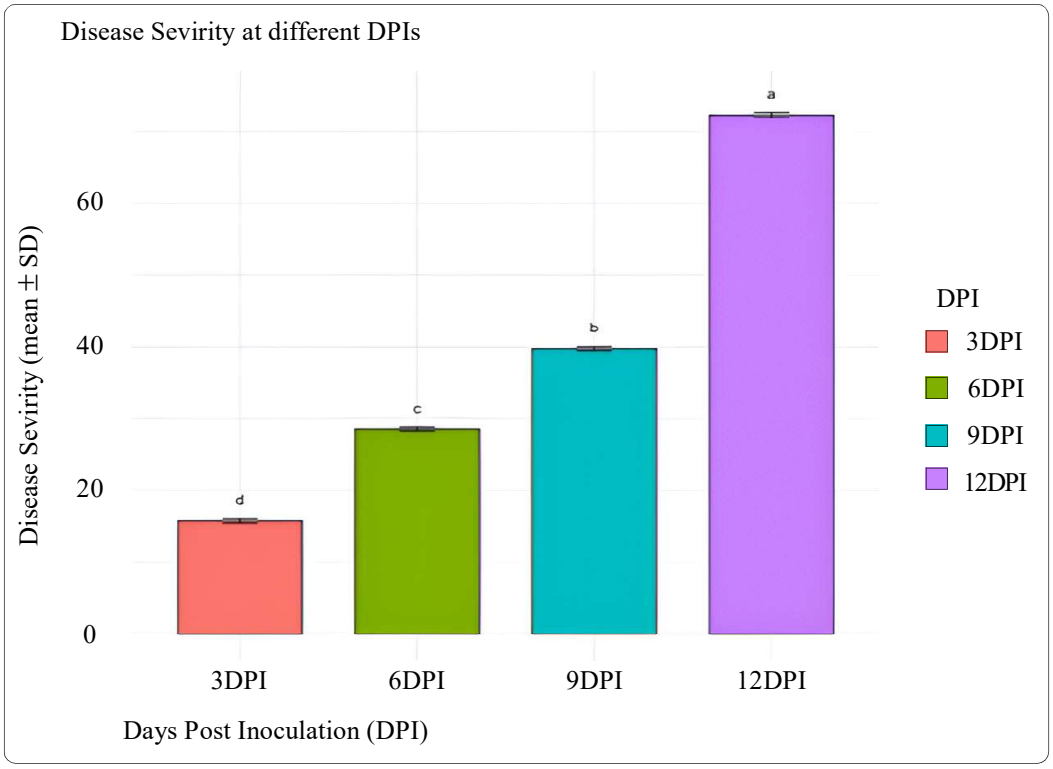

Fig. 5 : Disease Severity of *Shivam* at Different Days Post Inoculation

with the critical phase of lesion expansion and widespread tissue necrosis, characteristic of bacterial leaf spot in susceptible genotypes. Similar progression patterns have been reported in Srivastava *et al*., 2021 and Jones *et al*., 1991, where the absence of effective resistance mechanisms allowed the pathogen to multiply resulting in severe foliar damage. These findings confirm the highly susceptible nature of the Shivam cultivar and highlight the capacity of *X. euvesicatoria* to cause significant tissue destruction in vulnerable hosts.

Bars represent mean disease severity (± SD) of Shivam at 3, 6, 9 and 12 days post inoculation (DPI). Different letters above bars indicate significant differences among means according to Duncan's Multiple Range Test (DMRT) at $p \geq 0.0$

Bacterial Colonization in Shivam Cultivar

The bacterial population of *Xanthomonas euvesicatoria* in the susceptible tomato cultivar Shivam increased consistently with time. At 3 DPI, the mean bacterial load was 6.1 × 10v CFU/ml, which rise to 9.67 × 10v CFU/ml at 6 DPI and 1.38 × 10w CFU/ml at 9 DPI. The highest population was recorded at 12 DPI with 1.88 × 10w CFU/ml, indicating a clear progression of bacterial multiplication within host tissues (Fig.6). The Histochemical staining of the infected tissues shows a gradual increase in live cells (green fluorescence) over time, while the presence of only a few dead cells (red fluorescence) suggests a compatible interaction, which is further supported by bacterial proliferation confirmed through colony-forming unit analysis (Fig.7). This increasing trend shows the aggressive colonisation capacity of *X. euvesicatoria* in a susceptible cultivar. Comparatively, low variation at early time points of infection points to uniform bacterial establishment, while the greater variability observed at 12th DPI reflects differences in lesion expansion and tissue colonisation as the infection progressed. Similar population dynamics have been reported in Varympopi *et al*.,2022, where susceptible tomato lines supported

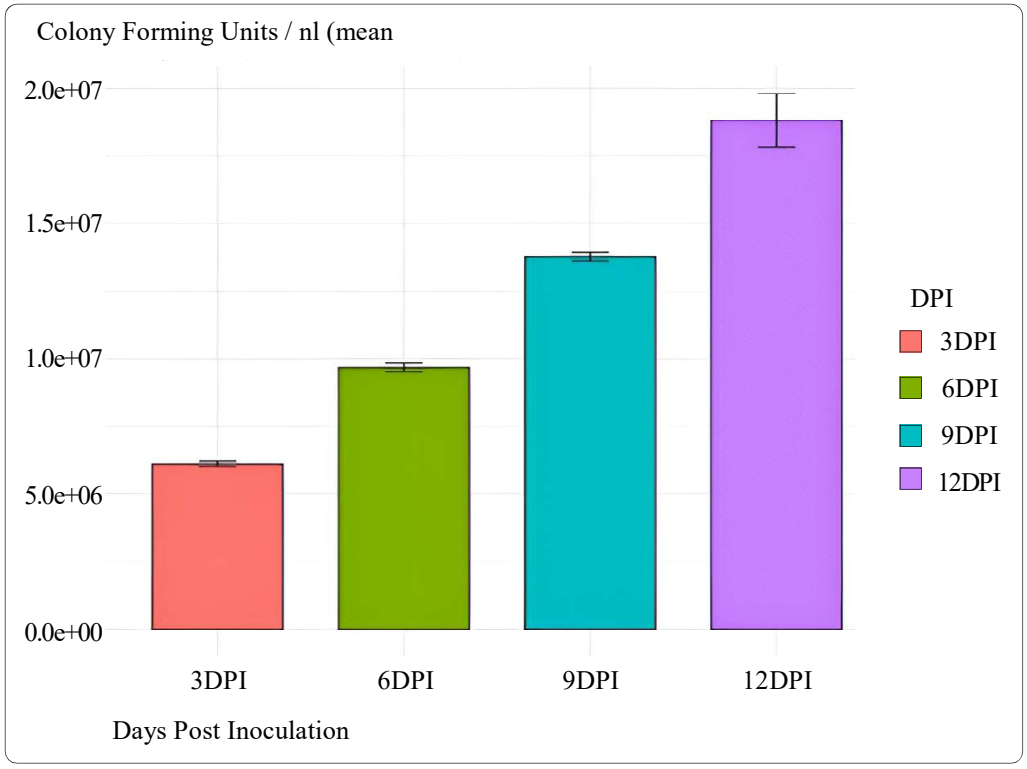

Fig. 6 : Colony Forming Units of Shivam at Different Days Post Inoculation

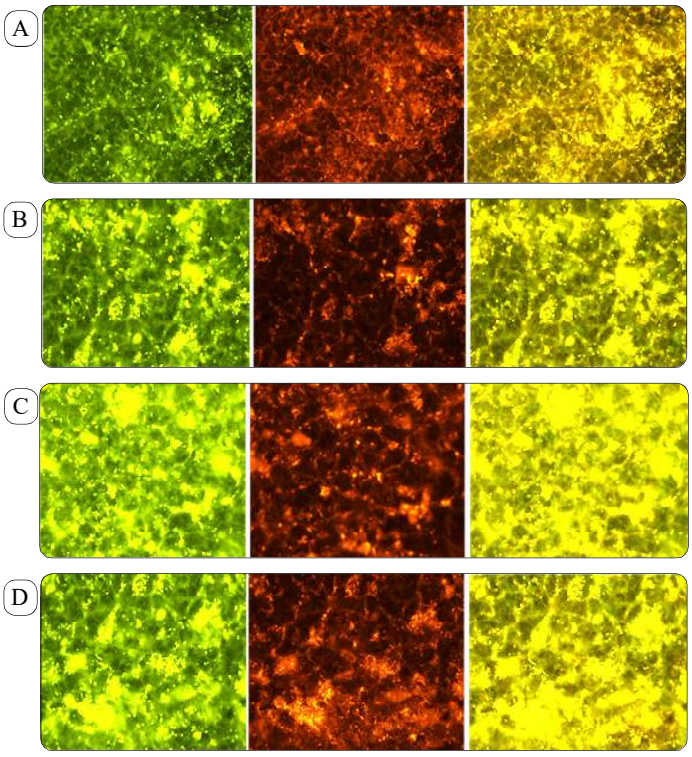

Fig. 7 : Histochemical Staining of Infected Leaf Tissue in *Shivam* at 12 Days Post Inoculation (DPI)

exponential bacterial growth that ultimately resulted in severe disease development. These findings highlight the importance of bacterial population buildup in assessing disease severity and progression. In contrast to resistant cultivars, where bacterial multiplication is often restricted, Shivam permitted continuous pathogen proliferation, confirming its high susceptibility. These findings point to the necessity of adopting effective monitoring and management strategies to limit the impact of bacterial leaf spot under field conditions.

Bars represent mean colony forming units per millilitre (CFU/ml) ± standard deviation (SD) of Shivam at 3, 6, 9 and 12 days post inoculation (DPI). Different letters above bars indicate significant differences among means according to Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

Histochemical staining of infected leaf tissue showing bacterial colonization in *Shivam* at different days post inoculation (DPI): (a) 3 DPI, (b) 6 DPI, (c) 9 DPI, and (d) 12 DPI.

This study conclusively identified *Xanthomonas euvesicatoria* as the causal agent of bacterial leaf spot in tomato at the University of Agricultural Sciences, GKVK campus, Bengaluru. The integration of morphological traits, biochemical reactions and 16S rRNA-based molecular characterization provided reliable confirmation of pathogen identity. Pathogenicity assays showed that the susceptible cultivar Shivam supported continuous pathogen multiplication, with disease severity rising sharply between 9 and 12 DPI and bacterial populations reaching 1.88 × 10w CFU/ml. These results highlight the aggressive nature of *X. euvesicatoria* and its capacity to cause significant foliar damage in susceptible cultivars. The study provides insights into pathogen biology, colonization patterns and virulence, forming a valuable basis for epidemiological studies and future management strategies. The continuous monitoring of pathogen diversity and population dynamics will be essential to mitigate the impact of bacterial leaf spot in tomato production systems.

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Endophytic Bacteria Associated with Browntop Millet (*Brachiaria ramosa*): Isolation, Plant Growth Promotion Traits and Antagonistic Efficacy against Leaf Blight

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K. Nagaraju : Supervision and draft correction

K. Tamilvendan : Conceptualization and draft correction

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Abstract

Endophytic bacteria play a crucial role in promoting plant growth and enhancing stress tolerance, making them valuable candidates for sustainable agriculture. This study focuses on the isolation and characterization of endophytic bacteria from *Brachiaria ramosa* (browntop millet), a resilient cereal crop known for its adaptability to marginal soils and drought conditions. Healthy plant tissues including roots, stems, and leaves were surface-sterilized and cultured on nutrient-rich media to isolate bacterial endophytes. A total of 85 distinct bacterial isolates were obtained and subjected to morphological characterization and to evaluate for PGP activity and antagonistic assay against the pathogen *Bipolaris seteriae*. Forty isolates exhibited significant antifungal activity, with LEB-82 showing the highest inhibition (89.08%), followed by LEB-31 (85.60%), comparable to the positive control *Pseudomonas fluorescens* (84.90%). Functional assays demonstrated LEB-82 consistently exhibited superior plant growth-promoting traits such as HCN production, phosphate solubilization, siderophore synthesis and ammonia production. These findings highlight the potential of browntop millet associated endophytes, particularly LEB-82, as biofertilizers and biocontrol agents to improve crop health and productivity under sustainable cultivation systems. The study lays the groundwork for future applications of these endophytes in biofertilizers and biocontrol agents, contributing to sustainable millet cultivation and improved crop productivity.

Keywords : Endophytes, Browntop millet, Biocontrol, Pathogen

Millets, often referred to as '*nutri-cereals*', have historically played a vital role in traditional farming systems across Asia and Africa due to their adaptability, resilience and nutritional richness (Gomashe, 2017). Among these, browntop millet (*Brachiaria ramosa*), also known as *korale*, is a lesser-known yet highly resilient millet species that is emerging as a promising crop in sustainable agriculture. Native to the Indian subcontinent and parts of Southeast Asia, browntop millet is a short-duration, drought-tolerant cereal that thrives under poor soil and low-input conditions, making it an ideal candidate for cultivation in marginal environments (Mohanan *et al.,* 2025). Its rich nutritional composition, dietary fiber, iron, calcium and antioxidants has contributed to its rising demand in health-conscious and gluten-free food markets (Singh *et al.*, 2022). Endophytes are microorganisms primarily bacteria and fungi that reside within plant tissues without causing apparent disease. These symbionts colonize roots, stems, leaves and seeds forming mutualistic or commensal

associations with their host plants (Yadav and Yadav, 2017).

The growing evidence highlights their functional importance in plant biology, including enhancing growth, modulating hormonal pathways, improving nutrient uptake and conferring tolerance to abiotic stresses such as drought, salinity and heavy metals, as well as protection against biotic stresses including pathogens and pests (Tripati *et al.,* 2025). Notably, endophytes produce bioactive metabolites such as indole-3-acetic acid, siderophores and hydrolytic enzymes that facilitate nutrient cycling and disease suppression (Kumar *et al*., 2020). In recent years, endophytes have gained considerable attention as biofertilizers, biocontrol agents and stress-resilience enhancers, aligning with the goals of sustainable and climate-smart agriculture (Yadav *et al*., 2021).

Studies on finger millet, pearl millet and foxtail millet have demonstrated the potential of endophytes to improve crop productivity and disease resistance (Nandhini *et al*., 2018). However, despite the increasing nutritional and agricultural significance of browntop millet, its endophytic communities remain largely unexplored. This represents a critical gap, as its inherent stress tolerance and low-input requirements suggest the presence of a unique endophytic consortium that may contribute to its robustness (Vujanovic & Germida, 2017).

The isolation and characterization of endophytes typically involve surface sterilization of plant tissues followed by culturing on selective media, allowing for the recovery of cultivable bacterial and fungal isolates. Molecular and biochemical assays further aid in identification and functional evaluation, including traits such as phosphate solubilization, nitrogen fixation, phytohormone production and antifungal activity (Cosoveanu *et al.,* 2021). Harnessing such traits can reduce dependency on chemical fertilizers and pesticides, supporting eco-friendly agricultural practices. Therefore, the present study was undertaken to isolate, identify and functionally characterize endophytic microorganisms from browntop millet, with a focus on their potential role in plant growth promotion and biocontrol of leaf blight disease.

Material and Methods

Collection of Sample

The browntop millet plant samples were collected from different locations of Karnataka *i.e.,* ZARS, GKVK, Bengaluru, MARS, Raichur, KVK, Hagari, Bellary and ARS, Bheemarayanagudi, Shahapur.

Isolation of Endophytic Bacteria

The collected healthy plant samples were rinsed properly in running tap water to remove soil and dust particles. Leaf segments, stem, grains and roots were equally cut by sterilized scalpel, after which surface sterilization was done under laminar air flow. For eliminating surface microbes, washing of plant tissue with 70 per cent ethanol for 5 min, subsequently with 0.4 per cent sodium hypochlorite for 4 min, three to four times washing with sterile water (Bacon *et al.*, 2002). The surface sterilized samples were blot-dried using sterile filter paper and impregnated on nutrient agar plates in triplicate and plating was done from the final wash, which serves as the control. The plates were incubated at room temperature for 24 - 48 hours and pure cultures of endophytic bacteria were maintained on agar slants and glycerol stocks at -4°C for further experimental studies (Raveendra and Shivaprakash, 2018).

Morphological Identification of the Potential Bacteria

For phenotypic identification of selected endophytic bacteria based on the antagonistic activity against leaf blight pathogen, various morphological parameters (colony, shape and gram staining reaction) were recorded. The gram staining technique was performed to determine gram stain reaction of the isolates. A compound bright-field microscope at 100× magnification was used to view the gram stain slides (Photolo *et al.,* 2020).

Screening for Biocontrol Activity of Endophytic Bacteria against Leaf Blight Pathogen

Endophytic isolates were screened for their antagonist activity against leaf blight pathogen, (*Bipolaris seteria*) of browntop millet isolated from the diseased

samples collected from GKVK, Bengaluru. Both endophytic bacterial isolate and pathogen were inoculated on single potato dextrose agar (PDA) plate. The pathogen (5 mm diameter disc) was inoculated at the centre of PDA plate and 24-hour old culture of endophytic bacteria (108 cfu/ ml) was streaked at corner of the plate and incubated at 27 °C for four to eight days in triplicates. Observations were recorded when there was a full growth of pathogen in the control plate. The per cent inhibition on growth of the test pathogen was calculated using formula as suggested by Vincent (1927).

$$I = \frac{(C-T)}{C} \times 100$$

Where,

I = Per cent inhibition

C = Growth of fungal plant pathogens in control (mm),

T = Growth of fungal plant pathogens in dual culture plate (mm)

In vitro Screening of Endophytic Bacterial Isolates for Plant Growth Promotion and other Attributes

Phosphate Solubilization

For phosphate solubilization study, a single colony of each bacterial culture grown on NA plate was streaked on to Pikovskaya's agar plates containing tricalcium phosphate and incubated at 25± 2 °C for 7-10 days. The plates were analyzed for clear phosphate solubilization zone around the colonies. Phosphate solubilization was quantified by Phospho-molybdate blue colour method and the blue colour development was read in a spectrophotometer at 610 nm (Acevedo *et al*., 2014).

Qualitative Assay of Hydrogen Cyanide (HCN) Production

HCN production of bacterial endophytes was tested qualitatively following the method of Bakker and Schipper (1987). The antagonistic bacteria were streaked on NA medium amended with glycine at 4.4 g/l. Sterile filter paper saturated with picric acid solution (2.5 g of picric acid; 12.5 g of Na_2CO_3, 1000 ml of distilled water) was placed in the upper lid of the Petri plate. The dishes were sealed with Parafilm and incubated at 28 °C for 48 h. A change of colour of the filter paper from yellow to light brown, brown or reddish-brown was recorded as weak (+), moderate (++) or strong (+++) reaction, respectively.

Quantitative Assay of Hydrogen Cyanide (HCN) Production

Suspension (3 ml of 10^8 cfu/ml) of each bacterial isolate was inoculated in (250 ml) flask containing NA broth medium (100 ml) amended with glycine (4.4 g/l). Three replicates were used for each tested isolate. Non inoculated flasks were used as control. Strips of filter paper (10×0.5 cm) were soaked in alkaline picrate solution and kept hanging to the neck of the flask (one strip for each flask). The flasks were plugged and sealed off with Parafilm. Three replicates were used for each isolate. After incubation at 28 ± 2 °C for 4 days (with shaking at 140 rpm), the color of sodium picrate strips was changed to a reddish brown compound in proportion to the amount of HCN evolved. The color was eluted by placing the changed filter paper in a test tube containing 10 ml of distilled water, and its absorbance was read by the UV spectrophotometer at 625 nm. Distilled water was used to adjust the zero absorbance reading at 625 nm before taking reading (Reetha *et al.*, 2014 and Abd El-Rahman *et al.*, 2019).

Screening for Siderophore Production

The siderophore production capability of isolates was detected on Chrome azurol S (CAS) agar plates for qualitative assay. Isolates were grown initially in nutrient agar broth (NAB) for 24 hours on rotatory shaker with 150 rpm at 28°C ± 2 °C. Then 50µl of bacterial suspension was spotted on CAS agar plates and incubated for 4-5 days at 28±2 °C. Plates were observed for production of yellow-orange halo zone around the spot which indicates positive for siderophore production (Schwyn and Neilands, 1987).

Quantitative Estimation of Siderophore Production

Isolates found to be positive for siderophore production were screened quantitatively using CAS

liquid assay. After initial growth in NA, isolates were transferred to siderophore inducing medium (SIM) and incubated for 5 days on rotatory shaker with 200 rpm at 28±2°C. SIM was prepared as follows, Solution-1, 30.2 g of Piperazine-N,N'-bis (2-ethanesulphonic acid) buffer dissolved in 750 ml of salts solution containing KH_2PO_4 (0.3 g), NaCl (0.5 g) and NH_4Cl (1 g). Iron free solution-2 was prepared with Glucose (2 g), Mannitol (2 g), $MgSO_4.7H_2O$ (493 mg), $CaCl_2$ (11 mg), $MnSO_4.H_2O$ (1.17 mg), H_3BO_3 (1.4 mg), $CuSO_4.5H_2O$ (0.04 mg), $ZnSO_4.7H_2O$ (1.2 mg) and $NaMoO_4.2H_2O$ (1 mg) in 223 ml of distill water. Solutions 1 and 2 were then autoclaved at 15 lbs for 15 minutes. Solution 3 was prepared by dissolving casamino acid (10 % w/v) in distilled water, *i.e.,* 3 g in 27 ml of water and dissolved thoroughly on magnetic stirrer. The solution was filter sterilized using 0.2 micron membrane filter. Finally, solutions 2 and 3 were mixed into solution 1 and volume was adjusted for 1000 ml with pH 6.8. Following incubation of 5 days, cells were discarded by centrifugation at 7,000 rpm for 15 minutes. Resultant supernatant (0.5 ml) was mixed with the 0.5 ml CAS assay solution, 10 ģl of shuttle solution (2 M 5-sulfosalicyclic acid) was added. After 20 minutes of incubation at room temperature, absorbance was measured at 630 nm to estimate the loss of blue color to orange (Maheshwari *et al*., 2019). The percentage of siderophore units were calculated by the formula:

$$[(Ar - As)/Ar] \times 100$$

Where, *Ar* is the absorbance of reference sample at 630 nm (medium plus CAS assay solution and shuttle solution) and *As* is the absorbance of sample (supernatant plus CAS assay solution and shuttle solution)

Ammonia Production

Ammonia production was analyzed using the qualitative method of (Fouda *et al*., 2021). Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 mL peptone water and incubated for 72 h at 35 °C. Nessler's reagent (1 mL) was added to each tube as a colorimetric reagent for ammonia production. The color change to faint yellow indicated the minimum ammonia production, while the color change from deep yellow to a brownish color indicated the maximum ammonia production.

Quantification of ammonia production was done by Nesslerization spectrophotometric method. Two mL of the broth was taken in a eppendorf tube and centrifuged at 10,000 rpm for 5 min. Then 50 µl of Na-K-tartarate (25g/50ml) and 50 µl of Nessler's reagent were added sequentially to each tube containing the supernatant and the absorbance was measured at 450 nm (Mukherjee *et al.*, 2017).

Statistical Analysis

The observations collected on various parameters both in laboratory condition were subjected to appropriate statistical analysis. The analysis of variance and interpretation of data were done as per procedures given by Gomez and Gomez (1984). Means were separated according to Duncan Multiple Range Test (DMRT) and error bars in figures were explained with standard error, by using the Wasp 2.0 statistical tool.

Results and Discussion

Isolation of Endophytic Bacteria

A total of nine healthy browntop millet (*Urochloa ramosa*) samples were collected from different agro-climatic regions of Karnataka to isolate endophytic bacteria. From various plant tissues like roots, stems, leaves and grains, a total of 85 endophytic bacterial isolates were successfully obtained using surface sterilization techniques and nutrient media. The complete list of samples collected and source location is presented in Table 1.

The number and diversity of isolates varied across plant parts with root tissues yielding the highest number of isolates. This variation can be attributed to the distinct micro-environments and tissue-specific biochemical profiles that influence microbial colonization and endophyte community structure (Kushwaha *et al.*, 2020). The number of endophytes varies among plant parts because each tissue provides different nutrients, exposure and defense conditions;

Table 1

Details of browntop millet samples collected for isolation of endophytes

Sl. No	Place (District)	Location	Lattitude (N)	Longitude (E)
Sample 1	Bengaluru	ZARS	13.081387 æ%	77.541447 æ%
Sample 2		ZARS	13.007701 æ%	77.579004 æ%
Sample 3		ZARS	13.081387 æ%	77.541447 æ%
Sample 4		ZARS	13.007520 æ%	77.350012 º
Sample 5	Mandya	VC Farm, Research plot	16.204529 æ%	77.326589 æ%
Sample 6		Farmer Field	12.569237 æ%	76.818152 æ%
Sample 7	Bellary	Hagari, KVK	15.141912 æ%	77.063476 æ%
Sample 8	Raichur	MARS, UASR	16.120056 æ%	77.204036 æ%
Sample 9	Yadgir	CoA, Bheemarayanagudi	16.733852 æ%	76.799449 æ%

roots usually host the most due to direct soil contact and nutrient release, while leaves, stems and reproductive tissues harbor fewer due to stronger defenses and harsher environments. Higher number of endophytic bacteria indicates their possible contribution in promoting seed germination and establishment or modulating other physiological functions (Bhagyashree *et al.,* 2023). The complete list of isolates and their respective sources is presented in Table 2.

Morphological Characterization of the Endophytic Bacterial Isolates

Morphological characterization revealed considerable diversity among the isolates, encompassing both Gram-positive and Gram-negative bacteria, consistent with earlier findings in cereals such as finger millet and maize (Verma *et al.,* 2021). Results of the study are depicted in Table 3.

Table 2

Endophytic bacteria isolated from browntop millet

Sample. No	Isolate Name	No. of Isolates
Sample 1	GEB- 11, GEB- 12, GEB- 13, GEB- 14, REB- 11, REB- 12, REB- 13, REB- 14, LEB- 11, SEB- 11, SEB- 12	11
Sample 2	REB- 23, SEB- 21, SEB- 22, LEB- 21	4
Sample 3	LEB-31, LEB-32, SEB- 31, SEB-32, SEB-33, SEB- 34, REB- 31, REB- 32, GEB- 31, GEB- 32	10
Sample 4	LEB- 41, LEB- 42, REB- 41, REB- 42, SEB- 41	5
Sample 5	GEB-51, GEB- 52, GEB- 53, GEB- 54, SEB- 51,SEB- 52, SEB- 53, SEB- 54	8
Sample 6	LEB- 61, LEB- 62, LEB- 63, LEB- 64, LEB- 65, REB- 61, REB- 62, REB- 63, REB- 64, REB- 65, REB- 66, REB- 67, SEB- 61, SEB- 62, SEB- 63, GEB- 61	16
Sample 7	REB- 71, REB- 72, REB- 73, REB- 74, REB- 75, GEB- 71, GEB- 72, LEB- 71	8
Sample 8	LEB- 81, LEB- 82, LEB- 83, LEB- 84, LEB- 85, LEB- 86, SEB- 81, SEB- 82, SEB- 83, REB- 81, REB- 82, REB- 83, REB- 84	13
Sample 9	REB- 91, REB- 92, REB- 93, LEB- 91, LEB- 92, GEB- 91, GEB- 92, SEB- 91, SEB- 92, SEB- 93	10

Note : Note: F- Fungi, E- Endophyte, L- Leaf, R- Root, S- Stem, G-Grain

TABLE 3

Morphological characterization of endophytic bacterial isolates

Bacterial isolate name	Colony colour	Appearance	Cell shape	Grams reaction
GEB- 11	Yellow	Translucent, raised and smooth margin	Rod	Positive
GEB- 12	Light yellow	Round, raised, smooth margin	Cocci	Negative
GEB- 13	Light creamish	Round, raised, smooth margin	Rod	Positive
GEB- 14	White	Translucent, raised and smooth margin	Cocci	Positive
REB- 11	White	Flat, rough margin	Rod	Positive
REB- 12	Creamy white	Raised, smooth margin	Rod	Positive
REB- 13	Yellow	Raised, smooth margin	Cocci	Negative
REB- 14	Cream	Flat, irregular margin	Cocci	Negative
LEB- 11	White	Flat, rough margin	Rod	Positive
SEB- 11	Light yellow	Round, raised, smooth margin	Cocci	Negative
SEB- 12	Yellow	Raised, smooth margin	Rod	Positive
REB- 23	White	Translucent, raised and smooth margin	Cocci	Positive
SEB- 21	Yellow	Translucent, raised and smooth margin	Rod	Negative
SEB- 22	Cream	Raised, smooth margin	Cocci	Negative
LEB- 21	White	Raised, smooth margin	Cocci	Negative
LEB-31	Yellow	Raised, smooth margin	Rod	Negative
LEB- 32	Cream	Translucent, raised and smooth margin	Short rod	Negative
SEB- 31	Yellow	Raised, smooth margin	Rod	Negative
SEB- 32	Yellow	Raised, smooth margin	Cocci	Negative
SEB- 33	Yellow	Raised, smooth margin	Rod	Positive
SEB- 34	Yellow	Raised, smooth margin	Cocci	Negative
REB- 31	Cream	Translucent, raised and smooth margin	Cocci	Negative
REB- 32	Cream	Raised, smooth margin	Short rod	Negative
GEB- 31	White	Translucent, raised and smooth margin	Long rod	Positive
GEB- 32	White	Raised, smooth margin	Long rod	Positive
LEB- 41	Cream	Sticky, Raised, rough margin	Rod	Positive
LEB- 42	White	Sticky, Raised, rough margin	Rod	Positive
REB- 41	Cream	Translucent, raised and smooth margin	Cocci	Positive
REB- 42	Cream	Translucent, raised and smooth margin	Cocci	Negative
SEB- 41	White	Translucent, raised and smooth margin	Long rod	Positive
GEB-51	Yellow	Raised, smooth margin	Cocci	Negative
GEB- 52	Cream	Raised, smooth margin	Rod	Positive
GEB- 53	Yellow	Raised, rough margin	Rod	Positive
GEB- 54	Yellow	Flat, rough margin	Cocci	Negative
SEB- 51	White	Flat, rough margin	Rod	Positive

Continued....

TABLE 3 Continued....

Bacterial isolate name	Colony colour	Appearance	Cell shape	Grams reaction
SEB- 52	Yellow	Translucent, raised and smooth margin	Short rod	Positive
SEB- 53	Cream	Raised, smooth margin	Short rod	Negative
SEB- 54	Cream	Raised, smooth margin	Comma	Positive
LEB- 61	White	Translucent, raised and smooth margin	Cocci	Positive
LEB- 62	Cream	Flat, rough margin	Cocci	Positive
LEB- 63	White	Translucent, raised and smooth margin	Rod	Positive
LEB- 64	White	Flat, rough margin	Cocci	Negative
LEB- 65	White	Raised, smooth margin	Comma	Negative
REB- 61	Cream	Raised, smooth margin	Rod	Positive
REB- 62	Cream	Sticky, Flat, rough margin	Cocci	Negative
REB- 63	Yellow	Raised, smooth margin	Cocci	Negative
REB- 64	White	Raised, smooth margin	Cocci	Positive
REB- 65	White	Flat, rough margin	Rod	Positive
REB- 66	White	Flat, rough margin	Rod	Negative
REB- 67	White	Raised, smooth margin	Rod	Negative
SEB- 61	Cream	Sticky, Raised, rough margin	Rod	Positive
SEB- 62	White	Translucent, raised and smooth margin	Comma	Negative
SEB- 63	White	Sticky, Raised, rough margin	Cocci	Positive
GEB- 61	Creamish white	Flat, rough margin	Cocci	Negative
REB- 71	White	Raised, smooth margin	Rod	Positive
REB- 72	Cream	Flat, rough margin	Rod	Positive
REB- 73	White	Raised, smooth margin	Cocci	Negative
REB- 74	White	Raised, smooth margin	Cocci	Positive
REB- 75	White	Flat, rough margin	Rod	Positive
GEB- 71	Yellow	Flat, rough margin	Rod	Negative
GEB- 72	Yellow	Raised, smooth margin	Cocci	Negative
LEB- 71	Yellow	Flat, rough margin	Rod	Positive
LEB- 81	White	Raised, smooth margin	Rod	Positive
LEB- 82	Yellow	Flat, smooth margin	Rod	Negative
LEB- 83	White	Flat, rough margin	Rod	Positive
LEB- 84	Cream	Flat, rough margin	Rod	Positive
LEB- 85	White	Raised, smooth margin	Cocci	Positive
LEB- 86	Cream	Raised, smooth margin	Rod	Positive
SEB- 81	Cream	Raised, smooth margin	Rod	Positive
SEB- 82	White	Sticky, flat, rough margin	Cocci	Positive
SEB- 83	Yellow	Translucent, raised and smooth margin	Rod	Negative

Continued....

TABLE 3 Continued....

Bacterial isolate name	Colony colour	Appearance	Cell shape	Grams reaction
REB- 81	Cream	Raised, smooth margin	Rod	Negative
REB- 82	White	Raised, smooth margin	Cocci	Negative
REB- 83	White	Translucent, raised and smooth margin	Long rod	Positive
REB- 84	Yellow	Raised, smooth margin	Cocci	Positive
REB- 91	White	Raised, smooth margin	Rod	Positive
REB- 92	Cream	Raised, smooth margin	Cocci	Positive
REB- 93	White	Flat, rough margin	Long rod	Positive
LEB- 91	White	Flat, rough margin	Rod	Positive
LEB- 92	Cream	Raised, smooth margin	Rod	Negative
GEB- 91	Cream	Flat, rough margin	Cocci	Negative
GEB- 92	White	Raised, smooth margin	Cocci	Positive
SEB- 91	White	Flat, rough margin	Rod	Positive
SEB- 92	White	Raised, smooth margin	Rod	Positive
SEB- 93	Cream	Flat, rough margin	Rod	Negative

Biocontrol Activity of Endophytic Bacteria against Fungal Pathogen

All 85 endophytic isolates were screened for their ability to inhibit *Bipolaris setariae*, the causative agent of leaf blight in browntop millet, using the dual culture assay. The zone of inhibition was measured and the percentage inhibition was calculated relative to the untreated control.

Forty isolates exhibited significant antagonistic activity against *Bipolaris setariae*, the causal agent of leaf blight in browntop millet. Among these, LEB-82 (*Pseudomonas putida* GKVK-2) displayed the highest inhibition (89.08%), followed by LEB-31 (*Pseudonmonas chlororaphis* GKVK-1) (85.60%), which was comparable to the inhibition caused by the positive control *Pseudomonas fluorescens* (84.90%). The antifungal activity of these isolates is likely mediated by the production of antibiotics, siderophores, HCN and volatile inhibitory compounds, which disrupt fungal growth and metabolism. Similar observations have been reported in rice and wheat endophytes, where *Bacillus* and *Pseudomonas* spp. significantly suppressed fungal pathogens through multiple antagonistic mechanisms (Zhao *et al.*, 2018). The suppression of mycelial growth of fungal pathogen by bacterial endophytes may be due to the production of inhibitory allelochemicals, volatile and non volatile compunds, hydrogen cyanide and cell wall degrading enzymes by antagonists (Raveendra and Shivaprakash, 2018). These results highlight the strong potential of browntop millet endophytes as biocontrol agents against foliar pathogens. These results highlight the strong potential of browntop millet endophytes as biocontrol agents against foliar pathogens. The detailed results of the antagonistic activity are represented in (Fig. 1 and Table 4).

P Solubilization

In the preliminary qualitative test of the efficient fourty bacterial isolates 20 isolates solubilized phosphate with the solubilizing index ranging from 1.65 to 3.10, the highest solubilization was observed by SEB-11 followed by LEB-82. The quantitative analysis of phosphate solubilization of the isolates

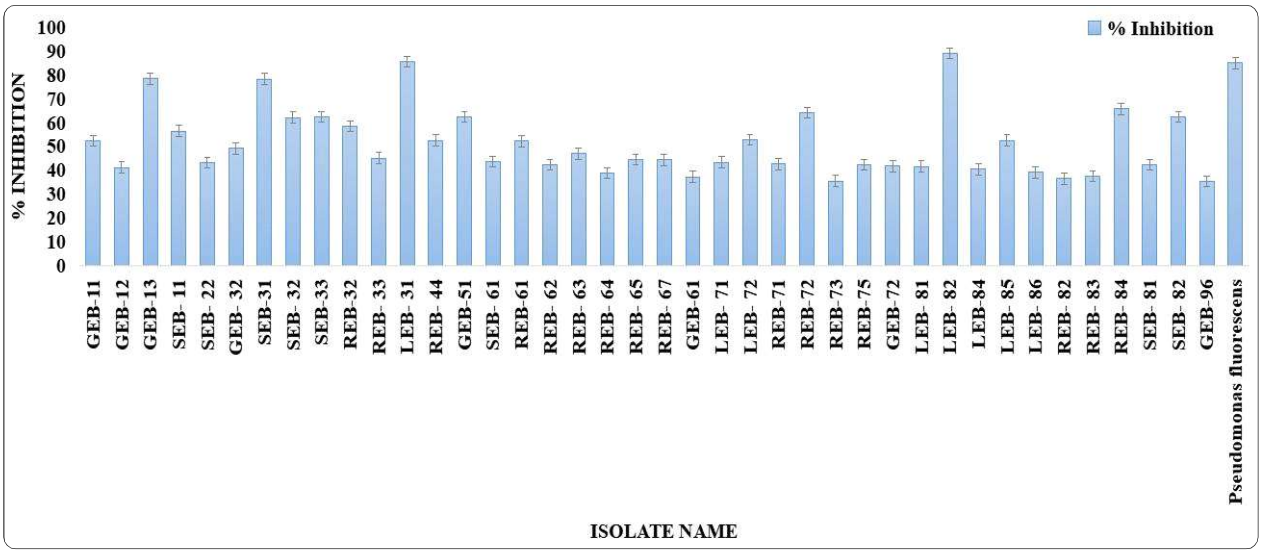


Fig. 1 : Biocontrol activity of bacterial endophytes in dual culture assay against *Bipolaris seteriae*

TABLE 4

Screening of the endophytic bacterial isolates *in-vitro* against *Bipolaris seteriae* by dual culture

Bacterial Isolate	% Inhibition
GEB-11	52.25 ^{i}
GEB-12	41.04 ^{r}
GEB-13	78.38 ^{c}
SEB- 11	56.43 ^{h}
SEB- 22	43.17 ^{no}
GEB- 32	49.08 ^{j}
SEB-31	78.26 ^{c}
SEB- 32	62.07 ^{f}
SEB-33	62.37 ^{f}
REB-32	58.39 ^{g}
REB- 33	45.06 ^{l}
LEB- 31	85.60 ^{b}
REB- 44	52.48 ^{i}
GEB-51	62.24 ^{f}
SEB- 61	43.55 ^{mn}
REB-61	52.09 ^{i}
REB- 62	42.2 ^{pq}
REB- 63	46.90 ^{k}
REB- 64	38.66 ^{t}
REB- 65	44.38 ^{l}
REB- 67	44.27 ^{lm}
GEB-61	37.12 ^{uv}
LEB- 71	43.28 ^{n}
LEB- 72	52.66 ^{i}
REB-71	42.46 ^{op}
REB-72	64.13 ^{e}
REB-73	35.41 ^{w}
REB-75	42.18 ^{pq}
GEB-72	41.64 ^{pqr}
LEB- 81	41.55 ^{qr}

Note : Means with same superscript, in a column do not differ significantly at P = < 0.05 as per DMRT

which have showed positive solubilization revealed significant results. The highest solubilization was recorded by SEB-11 followed by LEB-82 (Plate 3 (A) and Table 5).

Among the effective isolates, 20 demonstrated phosphate solubilization with indices ranging from 1.65 to 3.10. The highest solubilization was observed in SEB-11 (3.10) followed by LEB-82 (2.97). Quantitative assays further confirmed SEB-11 and LEB-82 as the most efficient

TABLE 5

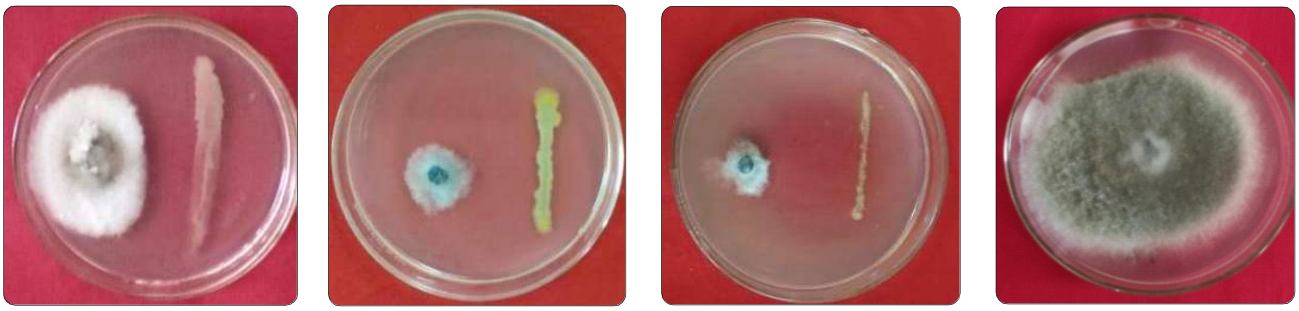
***In vitro* assay of bacterial isolates for plant growth promotion (PGP) and other attributes**

Bacterial isolate code	Phosphate solubilization index	Phosphate solubilization (µg/mL)	Ammonia production	Ammonia Production (mg/L)	Siderophore production	Siderophore Production (%)	HCN production	HCN production (mg/L)
GEB-11	2.76	9.84 ^{c}	-	0.00 ^{h}	++	53.67 ^{g}	+	0.015 ^{g}
GEB-13	1.68	5.12 ^{f}	++	3.42 ^{c}	++	57.55 ^{ef}	++	0.034 ^{ef}
SEB- 11	3.10	14.75 ^{a}	+	1.33 ^{g}	+	53.91 ^{g}	-	0.000 ^{h}
GEB- 32	1.75	7.75 ^{d}	+	1.75 ^{g}	++	70.14 ^{b}	+	0.014 ^{g}
SEB-31	2.68	10.73 ^{c}	+++	3.94 ^{b}	+	55.08 ^{fg}	-	0.000 ^{h}
SEB- 32	1.65	6.02 ^{e}	+	1.15 ^{g}	++	56.74 ^{f}	++	0.064 ^{cd}
SEB-33	0.00	0.00 ^{g}	-	0.00 ^{h}	+++	69.52 ^{bc}	++	0.059 ^{cd}
REB-32	0.00	0.00 ^{g}	+	2.19 ^{f}	++	56.86 ^{f}	-	0.000 ^{h}
LEB- 31	2.87	12.88 ^{b}	+++	4.08 ^{a}	+++	65.61 ^{cd}	+++	0.113 ^{b}
REB- 44	2.30	12.68 ^{b}	+	1.61 ^{g}	+	63.38 ^{de}	-	0.000 ^{h}
GEB-51	2.22	8.92 ^{d}	+	1.98 ^{g}	+	55.91 ^{fg}	++	0.036 ^{ef}
REB-61	2.13	11.95 ^{b}	++	3.01 ^{d}	+++	64.18 ^{de}	-	0.000 ^{h}
REB- 63	0.00	0.00 ^{g}	+	2.52 ^{e}	++	65.38 ^{cd}	-	0.000 ^{h}
REB- 65	0.00	0.00 ^{g}	+	1.31 ^{g}	+	54.02 ^{g}	-	0.000 ^{h}
LEB- 72	0.00	0.00 ^{g}	+	2.73 ^{e}	++	56.44 ^{f}	-	0.000 ^{h}
REB-72	1.82	7.02 ^{e}	++	3.28 ^{c}	+	57.61 ^{ef}	+++	0.079 ^{bc}
LEB- 82	2.97	13.62 ^{a}	+++	4.31 ^{a}	+++	76.34 ^{a}	++	0.169 ^{a}
LEB- 85	1.71	6.66 ^{e}	+	2.89 ^{d}	++	56.57 ^{f}	+	0.052 ^{de}
REB-84	1.97	5.49 ^{f}	+	2.37 ^{f}	++	53.01 ^{g}	++	0.064 ^{cd}
SEB- 82	2.61	10.82 ^{c}	-	0.00 ^{h}	+++	54.77 ^{fg}	+	0.028 ^{fg}
Pseudomonas sp.	2.95	13.65 ^{a}	+++	3.65 ^{b}	+++	69.98 ^{bc}	++	0.083 ^{bc}

Note : Means with same superscript, in a column do not differ significantly at P = < 0.05 as per DMRT

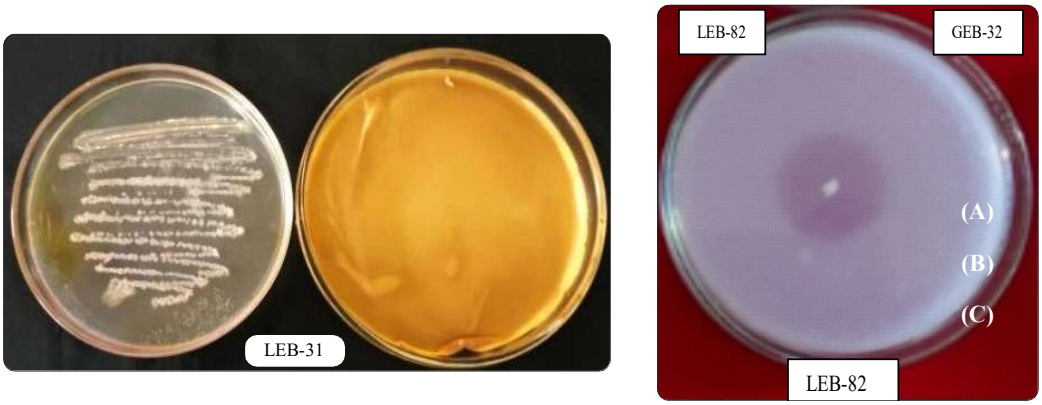
A. Leaf B. Stem C. Grains

Plate 1 : Isolation of endophytes from browntop millet plant parts



A. Isolate LEB-31 B. Isolate LEB-82 C. *Pseudomonas fluorescens* D. Control Pathogen

Plate 2 : Biocontrol assay of bacterial endophytes against pathogen



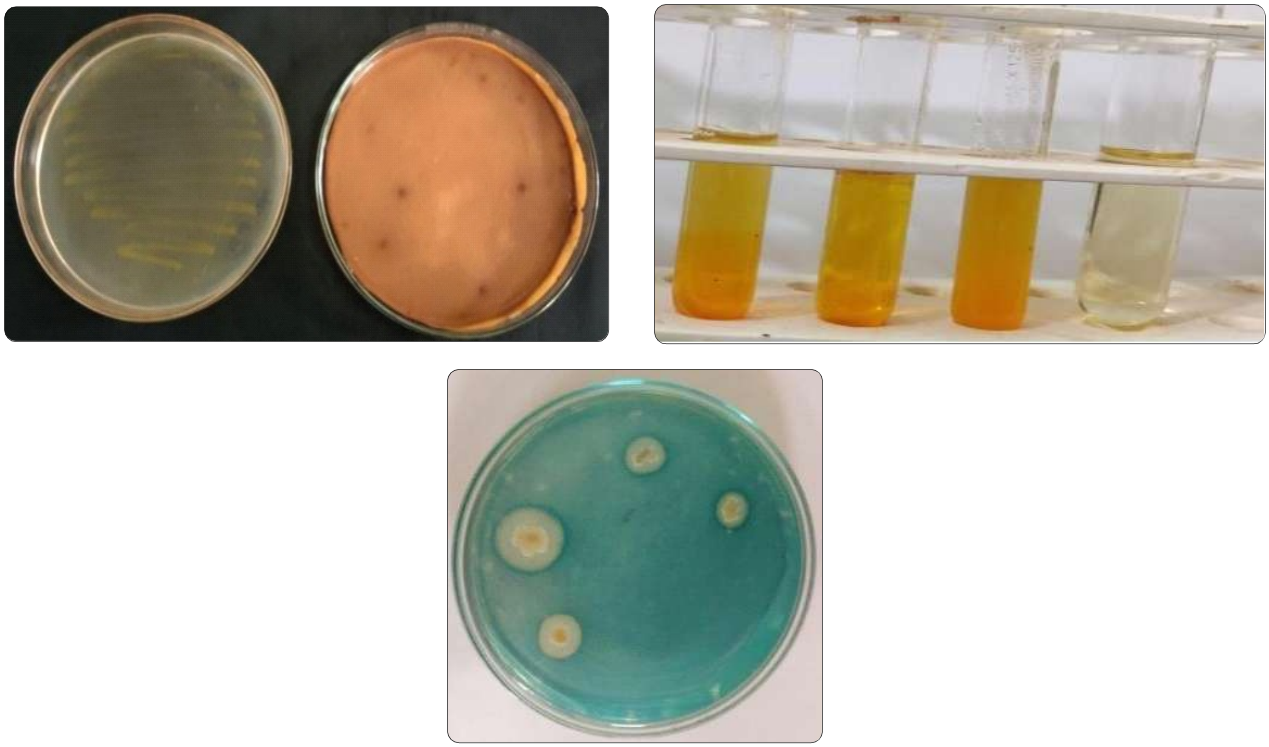


Plate 3 : Plant growth promotion activity of bacterial endophytes of browntop millet

(A) HCN Production, (B) PO_4^{-2} Solubilisation, (C) Ammonia production and (D) Siderophore production of bacteria

solubilizers, releasing 14.75 µg/mL and 13.62 µg/mL phosphate, respectively. Phosphate solubilization is a critical plant growth-promoting trait that enhances nutrient availability. Comparable findings were reported in pea endophytes, where *Enterobacter* and *Bacillus* spp. solubilized tricalcium phosphate, thereby improving phosphorus uptake (Tariq *et al.,* 2014). Thus, the phosphate solubilizing ability of browntop millet endophytes indicates their potential to reduce chemical fertilizer dependency.

Ammonia Production

Out of the 40 efficient endophytic bacterial isolates tested, 20 produced detectable levels of ammonia in peptone water medium, as confirmed by color change with Nessler's reagent Plate 3 (C). Quantitative analysis showed that ammonia production ranged from 1.15 mg/L to 4.31 mg/L. Among the isolates, LEB-82 exhibited the maximum production (4.31 mg/L), followed by LEB-31 (4.08 mg/L) presented in Table 5. Several other isolates, including REB-61 (3.01 mg/L) and SEB-31 (3.94 mg/L), also recorded significant ammonia production.

Ammonia production by bacterial endophytes is a key trait that contributes to plant nutrition and health. Ammonia serves as a readily available nitrogen source for plants and may enhance root and shoot development under nutrient-limiting conditions (Fouda *et al.,* 2021). Additionally, ammonia-producing bacteria are reported to indirectly suppress certain soil borne pathogens by creating localized toxic effects in the rhizosphere (Wani *et al*., 2007). Our findings align with earlier studies where endophytic *Bacillus* and *Enterobacter* species from chickpea and sorghum demonstrated similar ammonia-producing capacities, enhancing soil fertility and crop growth (Fouda *et al.,* 2021).

HCN Production

Hydrogen cyanide (HCN) production was detected in 12 out of 20 tested endophytic bacterial isolates. Quantitative assays revealed that LEB-82 produced the highest level of HCN (0.169 mg/L), followed by LEB-31 (0.113 mg/L) and REB-72 (0.079 mg/L). Several isolates showed moderate activity (0.034–0.064 mg/L), while others displayed weak or no detectable HCN production. The results are presented in the Plate 3(B) and Table 5.

HCN is a volatile secondary metabolite with strong antifungal and antibacterial properties. It is known to inhibit pathogen growth by interfering with cytochrome c oxidase and blocking the electron transport chain in fungal cells. The ability of browntop millet endophytes to produce HCN highlights their potential as biocontrol agents against foliar and soil borne pathogens. The present findings are similar with earlier reports where *Pseudomonas fluorescens* and *Bacillus subtilis* endophytes suppressed pathogens such as *Macrophomina phaseolina* and *Fusarium oxysporum* through HCN-mediated inhibition (Reetha *et al.*, 2014 and Abd El-Rahman *et al.*, 2019). The high HCN production by LEB-82, coupled with its strong antifungal activity against *Bipolaris setariae*, demonstrates a clear link between metabolite production and biocontrol efficiency.

Siderophore Production

In the qualitative screening, 20 isolates tested positive for siderophore production. Subsequent quantitative analysis revealed that LEB-82 produced the highest levels of siderophores, followed by GEB-32. The results are presented in Plate 3(D). The reference strain, included for comparison, showed 70 per cent siderophore production. Some isolates exceeded this value, indicating their superior capacity for iron chelation and potential to outcompete phytopathogens for iron in the rhizosphere.

Siderophore production was observed in 20 isolates, with LEB-82 producing the highest (76.34%), surpassing the reference strain (69.98%). Siderophores chelate iron, limiting its availability to pathogens and thereby restricting their growth. Results are presented in Table 5. The role of siderophores in pathogen suppression and growth promotion has been widely established in cereals (Maheshwari *et al.*, 2019). The high siderophore activity of LEB-82 suggests its strong competitive advantage in the rhizosphere and phyllosphere environments.

The present investigation establishes that browntop millet harbors diverse endophytic bacteria with significant plant growth-promoting and antagonistic properties. Among the 85 isolates, LEB-82 and LEB-31 demonstrated superior multifunctional traits, including phosphate solubilization, siderophore and HCN prouction, ammonia release and strong antifungal activity against *Bipolaris setariae*. These findings highlight the potential of endophytic bacteria as eco-friendly alternatives to chemical fertilizers and fungicides for improving the productivity and disease resistance of browntop millet.

In conclusion, the study underscores the role of browntop millet endophytes as promising bioinoculants that can be harnessed for integrated nutrient and disease management. Future work involving molecular identification, genome mining, metabolite profiling, and field validation of the most efficient isolates will be essential for translating these results into practical applications. The successful exploitation of such endophytes will not only enhance the resilience and productivity of browntop millet but also contribute to sustainable agricultural practices in stress-prone ecosystems.

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Marketing Channels of Cotton Ginning Mills in Raichur District of Karnataka

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ABSTRACT

Cotton a crucial agricultural commodity for India, plays an integral role in the nation's economy, contributing significantly to foreign exchange earnings, industrial production and export revenue. This paper examines the cotton marketing system, focusing on the ginning process and the efficiency of different marketing channels. The study specifically analyzes two primary channels in the cotton supply chain: Channel I, where cotton moves directly from farmers to ginning mills and then to spinning mills, and Channel II, where intermediaries (APMC traders) facilitate transactions between farmers and ginning mills. Data were collected from 15 cotton ginning mill owners in Raichur, Karnataka, to assess marketing costs, price spreads and marketing efficiency. The results indicate that Channel I is more advantageous for farmers, offering a higher net price and a greater share of the consumer rupee (35.45%) compared to Channel II (33.65%). The marketing efficiency, measured using Acharya's approach, is also higher in Channel I (0.55) compared to Channel II (0.51), demonstrating that a streamlined supply chain without intermediaries yields better economic outcomes for producers. The findings highlight the importance of reducing intermediaries to enhance producer returns and improve the overall efficiency of the cotton marketing system. This study provides valuable insights into optimizing marketing practices within the cotton industry to support sustainable agricultural development and improve the livelihoods of farmers.

Keywords : Cotton ginning mills, Marketing efficiency, Marketing channels, Price spread

COTTON, botanically known as *Gossypium hirsutum*, *Gossypium barbadense*, *Gossypium arboreum* and *Gossypium herbaceum*, is often called 'White Gold' due to its immense value. It is a vital fibre crop for India, playing a key role in both national and global economies. Cotton's natural qualities and versatility keep it dominant in the textile industry despite the rise of synthetic fibres. A growing preference for sustainable and eco-friendly materials has further bolstered its demand.

Cotton significantly contributes to India's net foreign exchange earnings. According to the Cotton Corporation of India, cotton exports totalled 3.0 million bales, valued at $5.66 billion in 2022-23, down from $10.78 billion in 2021-22. It accounted for 1.25 per cent of India's total exports in 2022-23. The exports include raw cotton, yarn, fabrics, garments and knitwear.

In Karnataka, cotton covers 6.86 lakh hectares, about 7 per cent of India's cotton area, with a production of 19.29 lakh bales (8% of national production) and a productivity of 478 kg per hectare. Major cotton-growing districts include Haveri, Raichur, Kalaburagi and Dharwad. Raichur, once known as the 'Cotton Bowl of Karnataka,' cultivates 1.73 lakh hectares, producing 0.42 lakh bales with a

productivity of 428 kg per hectare (Karnataka at a Glance Handbook, 2022-23). This cultivation supports both the local textile industry and the state's agricultural economy.

Ginning mills play a crucial role in the primary processing of cotton by separating the cotton fibers from the seeds, a process known as ginning. These mills serve as an important link between cotton farmers and the textile industry. The fiber extracted during ginning is called lint. The percentage of lint recovered from seed cotton is known as the ginning outturn ratio, which is typically around (33%). This lint is then sent to spinning mills, either within the state or to other states such as Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh or sometimes to bleaching units for further processing. Commission agents often facilitate these transactions. Additionally, ginning mills may directly export the lint or sell it to exporters involved in international trade. During the ginning process, approximately (65%) of the cotton seed is recovered from the seed cotton and is sent to nearby oil mills for cotton seed oil extraction.

In India, cotton ginning mills were extensive and predominantly located in rural and semi-urban cotton-growing regions, with approximately 4,135 mills in operation at the time. These mills were vital to the cotton-based industries, which contributed around 5 per cent to the country's GDP, (14%) to industrial production and (11%) to total export earnings, underscoring their significant role in the national economy (Anonymous, 2023).

Additionally, the strategic location of these mills in rural areas played a significant role in enhancing the local economy. These ginneries supported agricultural livelihoods and contributed to regional economic development by providing employment opportunities and fostering ancillary industries. The cotton ginning sector thus remains a vital element of India's agricultural and industrial landscape, reflecting both technological advancement and its impact on rural economic growth.

Intermediaries such as traders and commission agents play a vital role in cotton marketing. They are responsible for price determination and cleaning and separating foreign materials like bracts, leaves, stems and immature or unopened bolls. These intermediaries purchase seed cotton directly from farmers at the farm gate and then offer it for sale at local ginning mills or in mills located in other cities or states. During the 2021-2022 survey period, traders were particularly active due to a surge in cotton prices that exceeded the Minimum Support Price (MSP) set by the government. The Cotton Corporation of India Limited (CCI) is the primary government organization responsible for undertaking price support operations when cotton prices fall below the MSP. CCI purchases cotton from farmers at notified ginning mills or in designated Agricultural Produce Market Committee (APMC) yards. After purchasing the cotton, CCI delivers it to the ginning units for processing, covering the processing costs for the ginners and selling the resulting lint to spinning mills. Although several studies have examined cotton production and marketing efficiency in India, limited empirical evidence exists on the role of cotton ginning mills and the efficiency of alternative marketing channels in Raichur district of Karnataka. Earlier studies have largely focused on farmers or traders, with inadequate comparison of direct and intermediary-based channels using multiple efficiency measures. Moreover, detailed price spread analysis and systematic prioritization of constraints faced by cotton stakeholders remain under-explored. Hence, the present study attempts to bridge these gaps by analyzing marketing channels, efficiency, price spread and constraints in cotton ginning.

Methodology

The study was primarily relying on data from 15 cotton ginning mill owners from the Raichur. These mill owners served as sample respondents, providing essential information related to various aspects of their mills and the mode of marketing channel they were preferring. The required data were collected from those cotton ginning mills by personal interview method with the help of a pre-tested and well-structured schedule.

Table 1

Socio-economic characteristics of cotton Ginning Mill Owners in Raichur district

Characteristic	Category/ Range	Number of Mill Owners	Per cent (%)
Age (years)	< 35	3	20.0
	35 - 50	7	46.6
	> 50	5	33.4
Education Level	Up to Secondary	4	26.7
	Graduate & Above	11	73.3
Land Holdings (acres)	< 5	2	13.3
	5 - 15	8	53.4
	> 15	5	33.3
Family Size	< 5	6	40.0
	≥ 5	9	60.0
Annual Income (Rs.lakhs)	< 10	4	26.7
	10 - 30	7	46.6
	≥ 30	4	26.7

Ratio of Output to Input (Conventional Method)

ME = O / I x 100

Whereas :

ME = Index of marketing efficiency,

O = Value added,

I = Marketing cost

Value added = Difference between the price paid by the consumer to price received by the producers.

Shepherd's Method

Shepherd has suggested that the ratio of the total value of goods marketed to the marketing cost may be used as a measure of marketing efficiency. The higher the ratio, higher the marketing efficiency and vice versa (Shepherd 1965).

ME = CP / MC

Whereas :

ME = Index of marketing efficiency

CP = consumer's purchase price and

MC = Total marketing cost

Acharya's Method - Marketing Efficiency

MME = FP/ (MC +MM)

Whereas :

MME = Modified measure of index of marketing efficiency,

FP = Price received by farmer

MM = Marketing margin

MC = Total marketing cost

Price Spread

Price spread is the difference between the price paid by the consumer and the price received by the producer. It mainly consists of marketing costs and margins. The price spread analysis was carried out as follows:

$$\text{Producer's share in consumer's rupee} = \frac{\text{Producer's price}}{\text{Consumer's price}} \times 100$$

A fair marketing arrangement is extremely desirable in any economy. It promotes the interests of producers, consumers and governance with due importance. Remunerating farmers for their efforts, intermediaries for their service and consumers deriving value for the

money they paid have to be looked at alike. Such a marketing arrangement will be efficient. Acharya's approach was used to assess the marketing efficiency in different marketing channels followed by the cotton ginning mills in the study area. An Efficient marketing system plays an important role in increasing producers' share in consumers' rupee, which in turn encourages farmers/ firms to increase production. Spinning mills were considered as the consumers of ginned cotton in the present study.

Garrett Ranking Technique

This technique was used to study problem faced by the farmers in production and marketing of cashew and to rank those constraints faced by the farmers in the study area. Farmer respondents were asked to rank the constraints listed. In this analysis, rank one means most important constraint and last rank means less constraint. In the next stage, rank assigned to each constraint by each individual farmer was converted into per cent position using the following formula,

Garrett's formula for converting ranks into per cent was given by

$$\text{Per cent position} = 100\,\frac{(Rij - 0.5)}{Nj} \quad .....(5)$$

Where,

R_{ij} = rank given for i^{th} factor by j^{th} individual

N_j = number of factors ranked by j^{th} individual

The per cent position of each rank then converted into scores referring to the table given by Garret and Woodsworth (1969). For each factor, the scores of individual respondents were added together and divided by the total number of the respondents for whom scores were added. These mean scores for all the factors were arranged in descending order, ranks were given and most important factors were identified.

Marketing Efficiency of Major Marketing Channels in Cotton Ginning

The survey of cotton ginning mills during the study revealed two distinct channels employed by various mills in buying raw cotton to selling ginned cotton lint to the consumers *i.e.*, spinning mills. Channel I represented a direct and streamlined supply chain, where cotton moved directly from Farmers to Ginningmills for processing and then to Spinning mills without any intermediaries, ensuring a seamless flow from one stage to the next. In contrast, Channel II introduces complexity by including APMC traders as intermediaries between farmers and ginning mills. These traders, part of the Agricultural Produce Market Committee, handle the buying and selling of cotton, adding an extra layer to the supply chain that can affect pricing, timing and overall efficiency.

Results and Discussion

Table 2, indicates the marketing cost and price spread across different marketing channels. In analyzing the marketing costs and price spreads across two channels, Channel I proved more advantageous for farmers. In Channel I, farmers receive a price of Rs.7,800.00 per quintal, which, after accounting for total costs of Rs.1,181.20, yields a higher net price of Rs.6,618.80. In contrast, in Channel II, the sale price is lower at Rs.7,404.00 per quintal and although total costs remain the same, the net price received by farmers is reduced to Rs.6,222.80 per quintal. This difference highlights Channel I's superior benefit to farmers due to its higher sale price.

For APMC traders, the total cost incurred was Rs.7,634.00, which includes APMC charges, commission, transportation and loading/unloading costs. The margin achieved by traders is Rs.166.00 per quintal, reflecting the operational costs involved in managing the trade between farmers and ginning mills. This modest margin underscores the trader's role in facilitating the transaction while covering various operational expenses.

Ginning mills experience high processing costs of Rs.1,968.32 per quintal, along with additional expenses for packaging, transportation and loading/ unloading, bringing their total costs to Rs.9,873.89. Despite these high costs, ginning mills achieve significant margins due to the substantial markup from the purchase price of Rs.7,800.00 to the sale price of Rs.22,000.00. Their margins amount to Rs.12,126.11,

TABLE 2

Marketing cost and price spread across different marketing channels

(Rs. Per quintal)

Particulars	Channel - I	Channel - II
Farmer		
Price received	7,800.00	7,404.00
Cost of production of kapas	1,012.00	1,012.00
Transportation cost	82.60	82.60
Labour charges	86.60	86.60
Total cost	1,181.20	1,181.20
Net price received	6,618.80	6,222.80
APMC Trader		
Purchase price		7,404.00
APMC Charges		65.00
Commission		100.00
Transportation cost		50.00
Loading unloading		15.00
Total cost		7,634.00
Margins		166.00
Sale price		7,800.00
Ginning Mills		
Purchase price	7,800.00	7,800.00
Processing cost	1,968.32	1,968.32
Packaging	42.90	42.90
Transportation cost	48.67	48.67
loading unloading	14.00	14.00
Total cost	9,873.89	9,873.89
Margins	12,126.11	12,126.11
Sale price	22,000.00	20,000.00
Spinning Mills (consumers)		
Purchase price	22,000.00	22,000.00
Price Spread	14,200.00	14,596.00
Net Producer's share in consumer's price	35.45	33.65

Note : Channel-I: Farmers → Ginning mills → Spinning mills
Channel-II: Farmers → APMC Trader → Ginning mills → Spinning mills

demonstrating the value added through processing and the considerable profit realized from the high sale price.

Spinning mills, the final link in the supply chain *i.e.,* consumers for the ginned cotton show a larger price spread in Channel II of Rs.14,596.00 compared to Rs.14,200.00 in Channel I. This wider price spread in Channel II results in a lower net producer's share of (33.65%) compared to (35.45%) in Channel I. This indicates that consumers in Channel II benefit from a larger portion of the final sale price, reducing the share returned to producers.

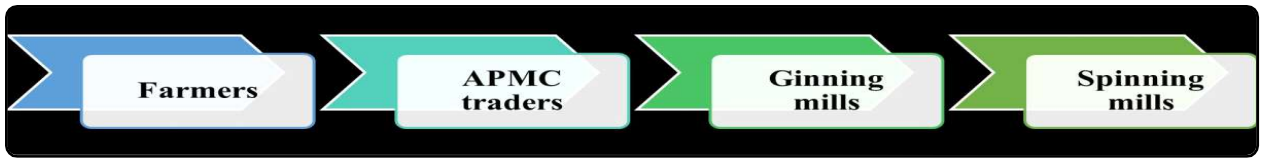
Overall, Channel I provided a higher net price for farmers and a better producer's share in comparison to Channel II, where higher costs and a larger price spread resulted in a diminished share for producers. This detailed analysis highlights the economic advantages and disadvantages of each marketing channel for different stakeholders in the supply chain, illustrating the impact of pricing and costs on overall financial outcomes.

The comparison of marketing channels presented in the table indicates that the difference between Channel I and Channel II is relatively small, which can be justified on the basis of the following observations. At the farmer level, the total cost of production remains identical in both channels (Rs.1,181.20 per quintal), as expenses on production, transportation and labour do not vary with the marketing route. Although the price received by farmers is slightly higher in Channel I (Rs.7,800) compared to Channel II (Rs.7,404), the resulting difference in net price received is only Rs.396 per quintal, indicating a marginal advantage of Channel I.

In Channel II, the presence of APMC traders introduces additional costs such as APMC charges, commission, transportation and loading/ unloading amounting to Rs.230 per quintal, with a trader margin of Rs.166 per quintal. However, these costs and margins are relatively small when compared to the overall value of the commodity and the margins earned by ginning mills. At the ginning mill level, the purchase price (Rs.7,800), processing cost (Rs.1,968.32) and total cost

Channel I

Channel II



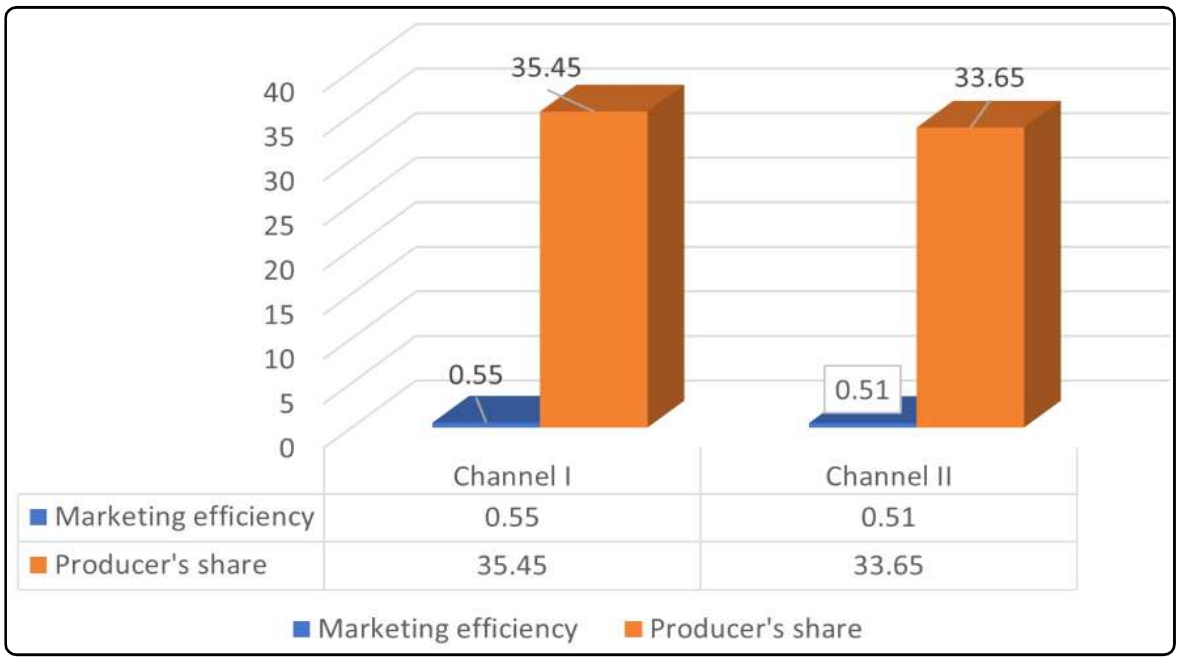


Fig. 1 : Producer's share and Efficiency of marketing channels involved in cotton ginning

(Rs.9,873.89) remain exactly the same in both channels. Further, the margin earned by ginning mills is also identical (Rs.12,126.11). Since ginning costs and margins constitute a major share of the total value addition, their similarity across channels limits the overall difference between the channels. At the consumer (spinning mill) level, the purchase price is Rs.22,000 per quintal in both channels. Consequently, the price spread differs only marginally, being Rs.14,200 in Channel I and Rs.14,596 in Channel II. This results in a small difference in the net producer's share, which is 35.45 per cent in Channel I and 33.65 per cent in Channel II.

Overall, the table clearly shows that major cost components and sale prices remain unchanged across the two channels and the additional costs introduced by intermediaries in Channel II are regulated and relatively small. Therefore, the difference in price spread and producer's share between Channel I and Channel II is not substantial, explaining why both channels exhibit similar marketing performance, with only a slight advantage in favour of Channel I.

TABLE 3

Efficiency of marketing channels involved in cotton ginning (Rs. Per quintal)

Particulars	Channel - I	Channel - II
Total marketing cost	2,073.89	2,303.89
Total marketing margin	12,126.11	12,292.11
Sale Price	22,000.00	22,000.00
Price Spread	14,200.00	14,596.00
Producer share in consumer rupee (%)	35.45	33.65
Marketing efficiency	0.55	0.51

TABLE 4

Breakup of marketing costs and margins in different channels (Rs. per quintal)

Particulars	Channel - I	Channel - II
Farmer costs	1,181.20	1,181.20
Trader costs	-	230.00
Ginning mill costs	2,073.89	2,073.89
Total marketing costs	2,073.89	2,303.89
Trader margins	-	166.00
Ginning mill margins	12,126.11	12,126.11
Total marketing margins	12,126.11	12,292.11

TABLE 5

Marketing efficiency by different methods in cotton ginning (Rs. per quintal)

Method of measuring marketing efficiency	Channel - I	Channel - II
Conventional method	685.08	590.90
Shepherd's method	10.61	8.91
Acharya's method (MME)	0.55	0.59

The Table 6 shows the results of Garrett Ranking analysis for constraints faced by cotton farmers in Raichur district. High processing cost emerged as the most significant constraint, with the highest mean score (77.2), indicating that the majority of respondents perceived it as the main bottleneck

TABLE 6

Garrett Ranking of Constraints Faced by Cotton Farmers

Constraint	Mean Score	Rank
High processing cost	77.2	I
Labour shortages	72.0	II
Price fluctuations	68.1	III
Quality inconsistency	64.5	IV
Transportation delays	61.3	V
Delayed payments	59.6	VI
Limited market access	56.8	VII
Regulatory issues	52.4	VIII

affecting cotton value chain efficiency. Labour shortages (72.0) and price fluctuations (68.1) followed closely, highlighting labour dependency and frequent price variation as critical concerns influencing farmers' operational stability and income levels.

Other notable issues included quality inconsistency (mean score 64.5), transportation delays (61.3) and delayed payments for produce (59.6), all of which can negatively impact the profitability and predictability of cotton farming activities.

Limited market access and regulatory issues, with mean scores of 56.8 and 52.4 respectively, were also reported as persistent-but less severe-barriers. The gradation in scores demonstrates that while all constraints play a role, their intensity and impact as perceived by respondents vary substantially.

The use of Garrett's Ranking technique ensures an objective prioritization by converting individual respondent rankings into standardized numerical scores; this allows more nuanced insights compared to simple frequency counts, especially when similar constraints are ranked differently by multiple respondents. It is evident from the results that addressing the top-ranked constraints-especially high processing costs, labour shortages and price instability-should be a priority for researchers, policymakers and stakeholders seeking to improve

cotton production and marketing efficiency in the Raichur district. This ranked list aids in formulating targeted interventions to mitigate these critical challenges and enhance the overall functioning of the cotton value chain.

Cotton remains a cornerstone of India's agricultural and industrial economy, symbolizing its significance as 'white gold'. Its journey from cultivation to spinning mills demonstrates a dynamic interplay of production, processing and marketing, each contributing to its immense economic impact. The analysis of marketing channels reveals that Channel I offers a more efficient and farmer-centric model, ensuring higher returns and a larger share of the consumer's price for producers. By minimizing intermediaries, Channel I reduces costs and enhances the overall efficiency of the supply chain.

The ginning sector not only bridges the gap between farmers and the textile industry but also significantly boosts rural economic development through employment and ancillary industries. As India emphasizes sustainable and efficient practices, the cotton supply chain, especially models like Channel I, stands out as a blueprint for maximizing producer benefits while supporting industrial needs. Such insights provide valuable guidance for stakeholders aiming to enhance both economic returns and the livelihoods of those involved in cotton cultivation and marketing.

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Prediction of Storage Life of Jasmine Flower Based on Quality Parameters with SVM Model

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Abstract

Flowers are among nature's most beautiful creations, symbolizing life, beauty and joy. Apart from their aesthetic appeal, they hold therapeutic, economic and environmental importance. Jasmine (*Jasminum multiforum* L.), one of the earliest cultivated fragrant flowers, is highly valued but suffers heavy postharvest losses due to its short shelf-life and quality deterioration. (Ali *et al*., 2023). The present investigation is conducted to study the role of different packaging materials in maintaining the freshness and storage life of jasmine flowers stored at 5°C, along with predictive modelling using a Support Vector Machine (SVM). Four packaging materials were tested *viz.*, bamboo basket with newspaper lining (P_0), Corrugated Fiber Board (CFB) with polypropylene lining (P_1), CFB with polyethylene lining (P_2) and nylon bag (P_3). Among them, P_2 (CFB with PE lining) proved to be the most effective. It consistently preserved flower quality, recording the highest freshness index of 59.28 on the 16th day, followed by P_1 (55.39). In contrast, P_0 and P_3 showed sharp declines to 26.24, resulting in the early termination of storage studies. Flower opening index was also lowest in P_2 (57.59), suggesting that it slowed down the physiological changes responsible for flower wilting and opening. Statistical analysis using ANOVA confirmed significant differences among treatments for freshness index ($p = 0.002$), with P_1 and P_2 showing clear advantages over P_0 and P_3. However, there was no significant difference in flower opening index ($p = 0.985$). To strengthen the findings, the SVM algorithm was applied for prediction. The polynomial kernel of SVM gave the best results, showing high correlation coefficients for both training and testing data, with minimal prediction errors. Storing jasmine flowers in CFB with PE lining, combined with predictive modelling using SVM, can significantly extend their shelf life, maintain quality and reduce postharvest losses.

Keywords : Jasmine flower, Shelf-life prediction, SVM model, Packaging material, Quality parameters, Postharvest management

Among the first fragrant flowers that humans have ever cultivated is the jasmine (*Jasminum* spp.). It has been suggested that the Persian word 'Yasmyn,' which means 'Fragrance' or 'Gift from God,' is the source of the English term 'Jasmine.' Many tropical and subtropical nations, including Afghanistan, Nepal, Iran and Central Asia, have ornamented their gardens with jasmine plants for a variety of uses. In India, jasmine is used in religious events, festivals and marital customs. It is mostly utilized for the extraction of essential oil, which India mostly exports to the United States, England, Holland, Sweden, Japan, Norway and the European Union (Jawaharlal *et al.*, 2013).

Owing to their perishable nature, flowers suffer significant post-harvest losses of between 30 and 50 percent. Quantitative losses are easier to evaluate than qualitative losses, such as the acceptance of fresh products by consumers. Because of incorrect postharvest management, quantitative losses arise along the whole supply chain (Anand & Barua, 2022). This may be more common in India than elsewhere, maybe as a result of ignorance about 'Post-harvest handling' of flowers. Poor infrastructure for post-harvest management (PHM) and commodity processing is the main contributor to post-harvest loss. The best way to reduce these losses through better supply chain management such as better handling, package, storage marketing and process cut flowers efficiently. It is interesting to note that the development of artificial intelligence (AI) technology and machine learning (ML) predictive models has created new opportunities in different sectors, such as agriculture. Optimization with the help of AI makes use of algorithms that can find the best possible solution to a complex dilemma, including finding out the best storage conditions and packaging materials to be used to retain quality of agricultural products (El-Mesery *et al*., 2022). ML tools operate through call functions that have the ability to learn and adapt, according to Deshpande *et al*. (2023). Smart data analysis is expected to become increasingly more prevalent as a vital instrument for technological advancement, given an ongoing rise in the amount of data that is becoming available (Moradi *et al*., 2020).

Support Vector Machines (SVM) is adapted for regression tasks using three kernels namely linear, polynomial and radial basis function (RBF) kernel. SVM is particularly effective when dealing with non-linear regression problems (Bhat *et al*., 2022). Implementation SVM for prediction includes data preparation, data pre-processing. Model selection, training, prediction and model evaluation. To examine the effectiveness of the model, various metrics are computed during the evaluation process, includes the RMSE (Root mean squared error) and R^2 (Co-efficient of determination) (Gene, 2021).

Material and Methods

Location of Study

The experimental location is at GKVK Bengaluru, at 130 05" N latitude and 770 34" E longitude. The centre is at an altitude of 924 meters above mean sea level. The annual rainfall ranges from 528 mm to 1374.4 mm with the mean of 915.8 mm. The mean temperature recorded was 26 ± 2 ^{0}C and RH was 80 ± 5%.

Packaging and Storage Condition

The jasmine flowers were harvested at flower budding stage in the early hours from the experimental plots of the University of Agricultural Sciences, Bangalore. They were then packed in bamboo baskets with newspaper lining (P_0), CFB with Polypropylene (PP) lining (P_1), CFB with Polyethylene (PP) lining (P_2) and nylon bags (P_3). The flowers were stored under controlled conditions, specifically at a temperature of 5 ± 2 °C with relative humidity of 85 ± 5 per cent for 20 days. Quality parameters of jasmine flower were assessed at an interval of 4 days throughout the period of 20 days.

Quality Parameters

The quality attributes of the flowers including freshness index and flower opening index were evaluated in triplicate.

Freshness Index (FI)

The number of flowers which retained freshness without exhibiting petal wilting and browning were usually observed using the following score and expressed as per cent of fresh flowers or freshness index (Nirmala and Reddy, 1993).

$$FI = \frac{(7xX1) + (6xX2) + (5xX3) + (4xX4) + (3xX5) + (2xX6) + (1 \times X7)}{(X1 + X2 + X3 + X4 + X5 + X6 + X7) \times 7} \times 100 \quad ...(1)$$

The score used for freshness index is illustrated as below

Freshness Index scores

Condition of flower	Score	Number of flower buds under respective score
Almost all buds turgid	7	X_1
Partial to half open flowers, turgid	6	X_2
Half to full open flowers, turgid	5	X_3
Partial to half open flowers, slightly wilted	4	X_4
Half to full open flowers, slightly wilted	3	X_5
Partial to half open flowers, fully wilted	2	X_6
Half to full open flowers fully wilted	1	X_7

Flower Opening Index (FOI)

The flower opening index is the number of flowers which are opened and it is measured by visual observation. If petals of the flowers open in a short period of duration, it decreases the storage life and market value of the flowers. Accordingly, the flowers were observed and flower opening index was calculated by following equation suggested by Karuppaiah *et al.* (2006).

$$FOI = \frac{(0xX1) + (1xX2) + (2xX3) + (3xX4)}{(X1 + X2 + X3 + X4) \times 4} \times 100 \quad ...(2)$$

The score used for flower opening index is given as below;

Flower opening index scores

Stage of flowers	Score	Number of flower buds under respective score
Unopened buds	0	X_1
Slightly opened	1	X_2
Half opened	2	X_3
Full opened	3	X_4

Predicting the Shelf Life of Jasmine flowers (*Jasminum multiforum* L) using SVM Model

The changes in jasmine flower quality parameters during storage were predicted using a Support Vector Machine (SVM) model developed in Python. In this study, SVM models with linear, polynomial and radial basis function (RBF) kernels were evaluated, with the regularization parameter 'C' optimized. A total of 186 hyper-parameter combinations were implemented using the *scikit-learn* library. Experimental data on freshness index (FI) and flower opening index (FOI) were used for model training, while independent test data sets were employed to evaluate the model's predictive reliability on previously unseen data.

The SVM model was constructed using three input variables: type of packaging material, storage duration, and quality parameters. Prior to model development, preprocessing of input data was carried out using normalization and discretization techniques. Normalization scaled the variables into a fixed range (typically [0,1]), ensuring uniform weightage and eliminating bias caused by differing data magnitudes. Discretization was applied to improve model efficiency under nonlinear data distributions by transforming continuous variables into discrete intervals (Naeimi *et al*., 2025). The mathematical

$$X'_i = \frac{x_i - x_{min}}{x_{max} - x_{min}} \quad ...(3)$$

$$W = \frac{x_{max} - x_{min}}{k} \quad ...(4)$$

formulations of these preprocessing techniques are provided in the subsequent equations.

In SVM model three kernel functions *Viz.*, linear, polynomial and gaussian were used to develop prediction model for jasmine flower. The mathematical parameters for each kernel were calculated using equations.

Linear Kernel :

The linear kernel is the simplest type of kernel function and is defined as the inner product of two vectors.

$$K(x, x2) = x^T x\ 2 \quad \ldots\ldots (5)$$

When the data is linearly separable it is frequently used. The formula for the linear kernel between two vectors, x and x', is:

Where, x = Flower opening index and x2 = Freshness index and both are input vectors

T= Transpose

$$K(x, x2) = (\gamma x^T x' + r)^d \quad \ldots\ldots (6)$$

Polynomial Kernel

The polynomial kernel is a more flexible version of the linear kernel that can handle non-linear relationships. It's defined as a function of the inner product of two vectors, raised to a certain power. The formula is:

Where,

γ (gamma) is a scaling parameter

r (r) is a constant term

d (d) is the degree of the polynomial

$$K(x, x') = \exp(-\gamma \|x - x'\|^2) \quad \ldots\ldots (7)$$

Radial Basis Function (RBF) Kernel

The Radial Basis Function (RBF) kernel, also known as the Gaussian kernel, is one of the most widely used kernels. It is particularly effective for non-linear data and creates a decision boundary that is a smooth curve. The RBF kernel measures the similarity between two vectors based on their Euclidean distance. The formula is:

Where:

γ (gamma) is a scaling parameter that determines the influence of a single training example.

$\|x - x'\|^2$ is the squared Euclidean distance between the vectors x and x′.

The parameters of the kernels were optimized using classical grid search method which defines the range of values for each hyperparameters (c or γ).

Prediction Accuracy of the Model

The accuracy of the machine learning models under study was evaluated by the five model evaluation techniques (Moriasi *et al*., 2015). Two evaluation indices were used in this study *viz*., coefficient of determination (R^2), Root Mean Square Error (RMSE).

R-squared (R^2) or Coefficient of Determination

R squared (R^2), also known as the coefficient of determination, is a statistical metric that quantifies the goodness of fit of a regression model by indicating

$$R^2 = \frac{\sum(\hat{y}i - \bar{y})^2}{\sum(yi - \bar{y})^2} \quad \ldots\ldots (8)$$

how much of the variation in the dependent variable is explained by the independent variable (s). It ranges from 0 to 1 (or 0% - 100%), where an R^2 of 1 (100%) implies perfect prediction-*i.e.*, the model accounts for all variability-while an R^2 of 0 indicates no explanatory power

Root Mean Square Error (RMSE)

The difference between values predicted by a model and actual values is computed using RSME (Root mean square error). It is a type of inaccuracy in the method used to gauge the accuracy and error rate of

$$RMSE = \sqrt{\frac{(\bar{y}_i - \bar{y}_i)^2}{n}} \quad \ldots\ldots (9)$$

any machine learning algorithm used to solve a regression problem.

RMSE is a square root of value collected from the mean square error function. It helps us plot a difference between the estimate and actual value of a parameter of the model. Using RSME efficiency of the model is easily measured.

Results and Discussion

Quality Parameters of Jasmine Flower

Effect of different packaging materials on freshness index at 5°C were observed and depicted in Fig. 1 Among the different packaging materials used freshness index of jasmine flower was recorded maximum in P_2 (CFB with PE lining) for all the storage period. On 16th day of storage maximum freshness index of 59.28 was observed in P_2 followed by P_1 (CFB with PP lining) which is 55.39. In contrast, freshness index of P_0 (Bamboo basket with newspaper lining) and P_3 (Nylon bag) reduced drastically from 100 to 26.24 at the end of 16th day and their shelf-life study was terminated due to spoilage. The control (P_0) showed a higher reduction in freshness index of 26.24 by the end of the 16th day.

Packaging jasmine flowers in CFB boxes has been found to maintain a higher percentage of fresh flowers compared to other packaging methods. Packaging was crucial in maintaining optimal humidity levels, which slowed down the process of evapo-transpiration and ensured a proper balance of CO_2 and oxygen concentrations, thereby slowing down respiration. This observation is consistent with the findings reported by Nirmala and Reddy, (1993). The results are in agreement with Nagaraja (1999) where treated tuberose flowers packed with 100-gauge polyethylene lining with no ventilation showed higher freshness index.

Effect of different packaging materials on flower opening index of jasmine flower at 5°C were observed and depicted in Fig. 2. Among the different packaging materials used flower opening index of jasmine flower was recorded minimum in P_2

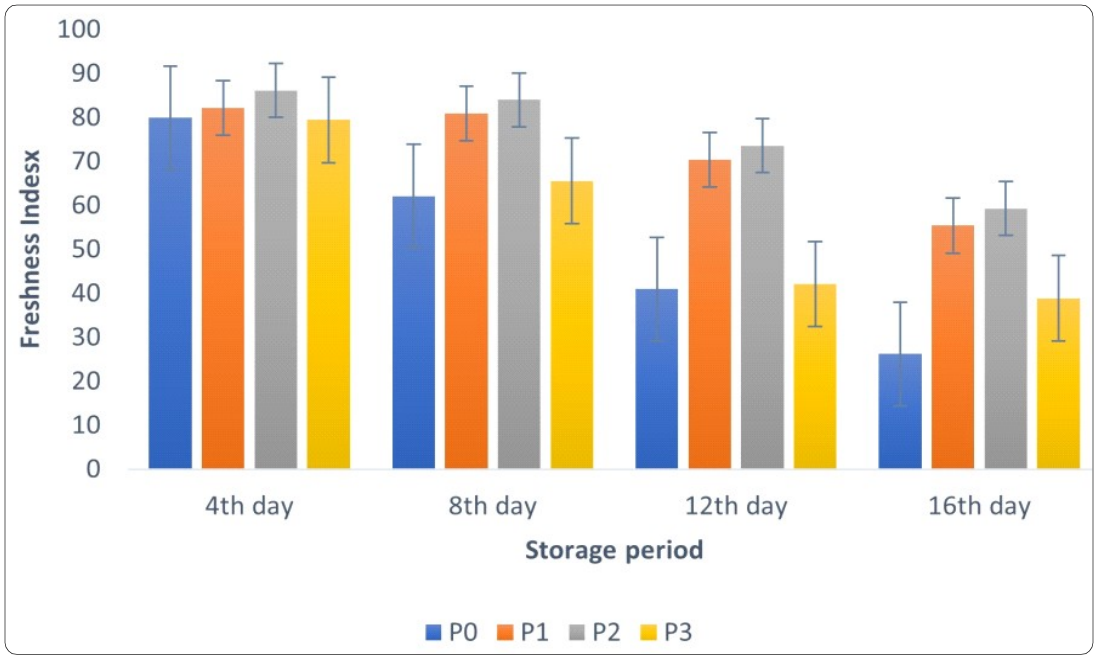

Fig. 1 : Effect of packaging material on Freshness index in jasmine flower during storage period at 5° C

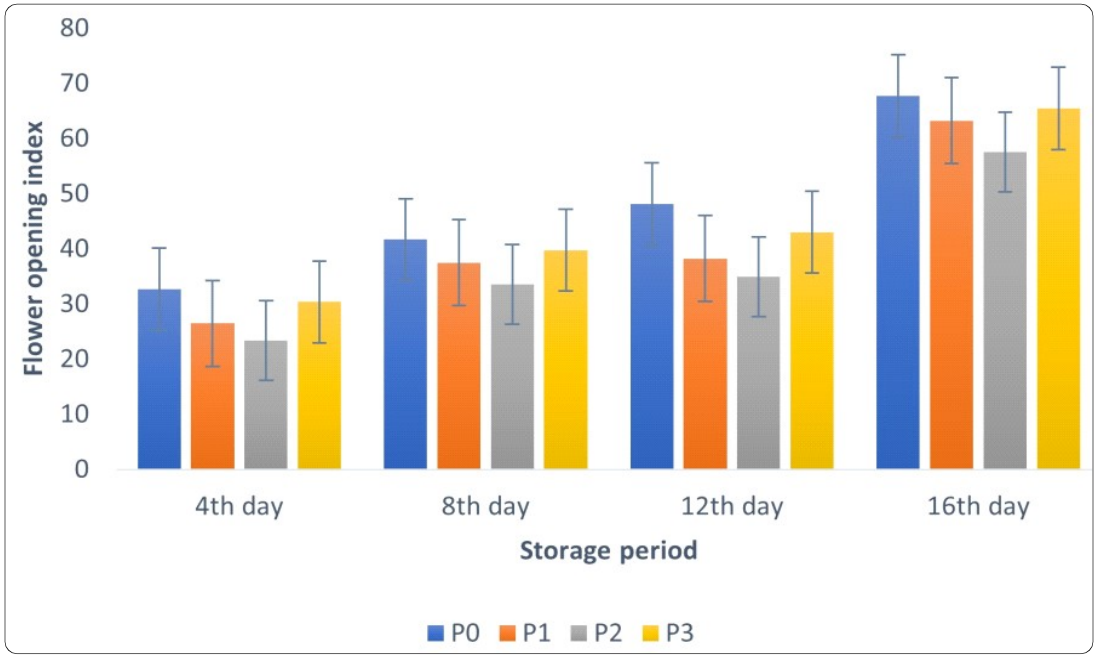

Fig. 2 : Effect of packaging material on Flower opening Index in jasmine flower during storage period at 5°C

(CFB with PE lining) for all the storage period. On 16th day of storage minimum flower opening index of 57.59 was observed followed by 63.22 in P_1. Flower opening index of P_0 (Bamboo basket with newspaper lining) and P_3 (Nylon bag) increases drastically till 16th day and later on their shelf-life study was terminated due to all flowers are fully opened. P_4 showed a higher increase in flower opening index from 0 to 67.74 by the end of the 16h day. Sudden increase in flower opening index was observed in all the packaging material till 16th day of storage. Later observation was discontinued due to termination of treatment and all flowers were fully opened.

Reduced water status of flowers is known to record the changes in flower opening under ambient conditions (Van Doorn and Witte 1991). Flower opening index were observed less with 200 gauge PE bag with no ventilation (Karuppaiah *et al*., 2006). The results were in agreement with Majumder *et al.,* 2014 which shows treating tuberose stored in PE bags kept at 4 °C can results in minimum flower opening index (10.33).

Implementation of SVM Algorithm

The SVM algorithm plays a crucial role in predicting storage life and modelling the studied parameters by providing a robust framework for handling complex, non-linear relationships in the data. Notably, Polynomial kernel outperformed the others in both Normalized and Discretize datasets (Table 1). Under training conditions, this kernel achieved the highest correlation coefficients of 0.97, 0.94 for freshness index and 0.96, 0.90 for flower opening index for both the Normalized and Discretize modes, respectively. Similarly, under testing this kernel outperformed with 0.96,0.90 for freshness index and 0.90, 0.94 for flower opening index respectively. This study showcases the effectiveness of SVM model in predicting shelf life of jasmine flower during storage period. Similar findings for color estimation and quality in agricultural products and food have been reported by Naeimi *et al*., (2025), Da Silva Cotrim *et al*., (2021).

The error plot for freshness index for different packaging material and storage time demonstrates that SVM model effectively model the freshness index

TABLE 1

Statistical results for predicting freshness index and Flower opening index in jasmine flower using SVM algorithm

Parameter	Data type	Kernel type	Training		Testing	
			R^2	RMSE	R^2	RMSE
Freshness Index	Normalize	Linear	0.73	2.01	0.65	2.66
		Polynomial	0.97	1.38	0.96	1.34
		RBF	0.88	1.96	0.79	2.11
	Discretize	Linear	0.79	3.61	0.77	3.85
		Polynomial	0.94	2.01	0.90	2.62
		RBF	0.91	2.83	0.88	3.23
Flower opening Index	Normalize	Linear	0.71	3.02	0.69	3.56
		Polynomial	0.95	1.82	0.90	1.89
		RBF	0.93	1.99	0.92	2.35
	Discretize	Linear	0.85	4.68	0.81	4.87
		Polynomial	0.96	1.23	0.94	1.42
		RBF	0.90	3.37	0.91	3.90

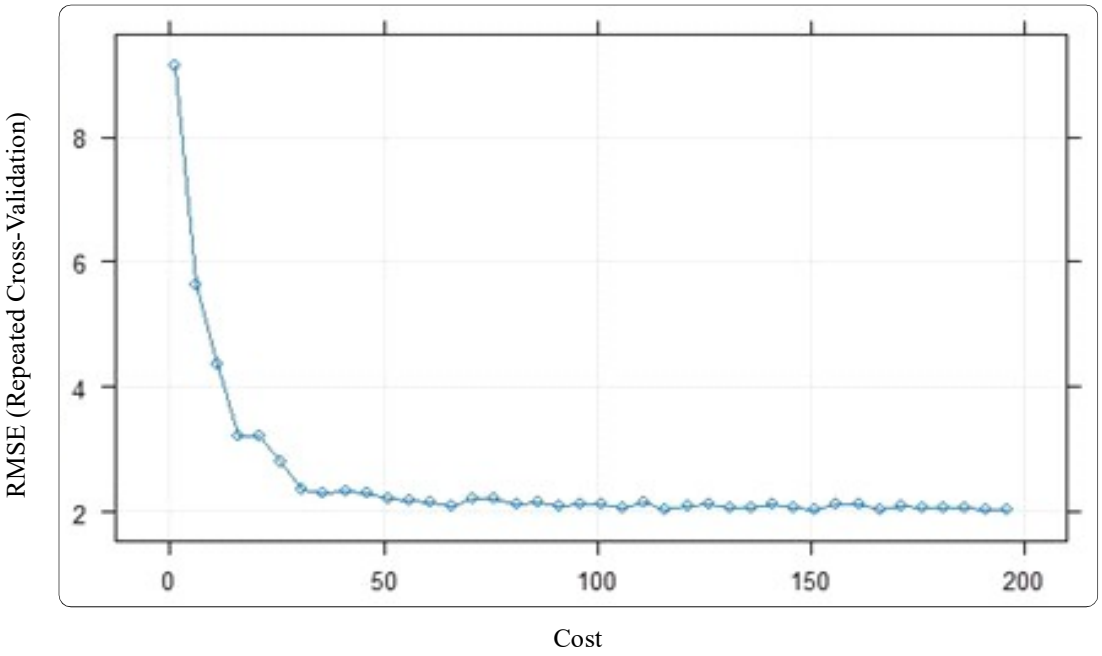

Fig. 3 : Error plot of SVM model for flower freshness index

when configured with an optimal number of cost value, as indicated by the lowest RMSE values. The model was tasked with minimizing the loss functions (RMSE) via an iterative approach. From the Fig. 3 it was observed that, the cost value of 166 exhibited the lowest RMSE values of 2.04 for SVM. A smaller loss function value indicates a better fit, as the predicted values are closer to the actual values.

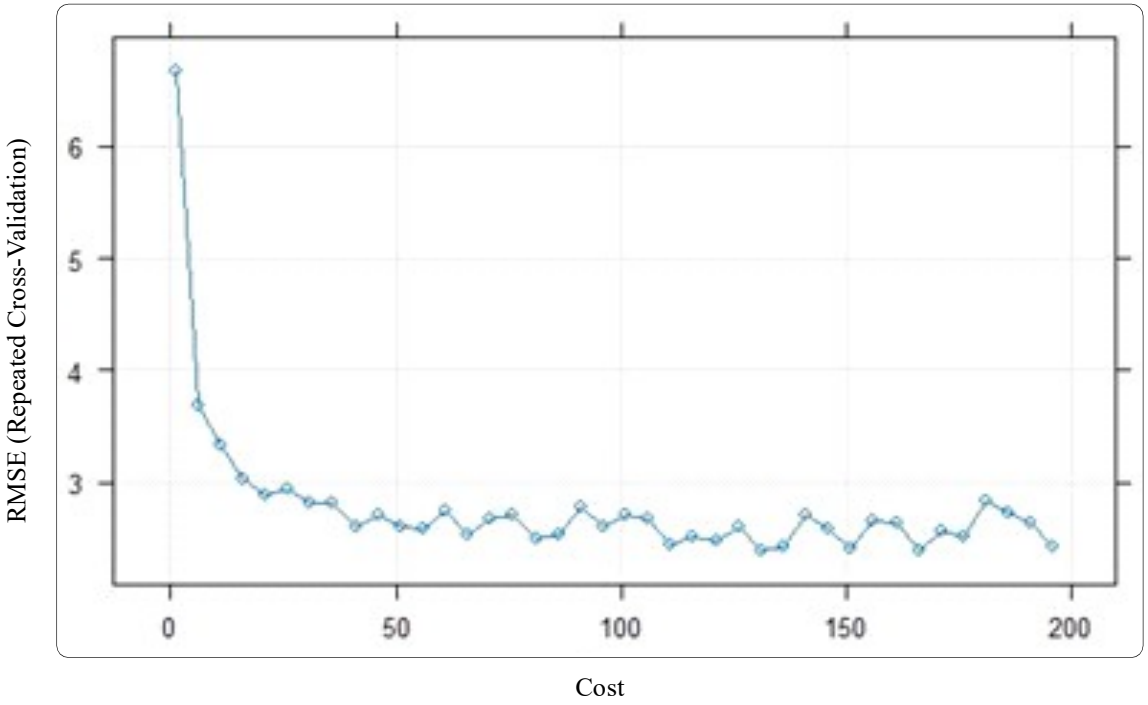

Fig. 4 : Error Plot of SVM Model for Flower opening index

The error plot for flower opening index for different packaging material and storage time demonstrates that SVM model effectively model the flower opening index when configured with an optimal number of cost value, as indicated by the lowest RMSE values. The model was tasked with minimizing the loss functions (RMSE) via an iterative approach. From the Fig. 4 it was observed that, the cost value of 166 exhibited the lowest RMSE values of 2.63 for SVM. A smaller loss function value indicates a better fit, as the predicted values are closer to the actual values.

The box plot of freshness index shown in Fig. 5 compares the freshness index of jasmine flowers for the treatments (P_0, P_1, P_2, P_3). P_0 and P_3 have lower means (≈46) with wide variability, indicating inconsistent freshness, while P_1 and P_2 show higher means (≈73-75) and narrower ranges, reflecting better and more consistent quality. Medians in P_1 and P_2 align closely with their means, suggesting symmetrical distributions. ANOVA results (F=5.849, p=0.002, N=49) confirm statistically significant differences between different treatments. Visually, P_1 and P_2 outperform P_0 and P_3 in maintaining freshness, implying these treatments or storage conditions were more effective in preserving quality. In contrast, P_0 and P_3 likely represent less optimal conditions, with greater variability and lower overall freshness values. Overall, the findings highlight that specific treatments can significantly improve and stabilize post-harvest freshness of jasmine flowers, which is crucial for extending shelf life and ensuring quality consistency in storage and distribution.

The box plot of flower opening index shown in Fig. 6 illustrates the variation in the flower opening index across four treatments (P_0, P_1, P_2, P_3). Each bar represents the mean value with standard deviation, highlighting the spread of data within treatments. P_0 recorded the highest mean flower opening index (27.11 ± 28.59), followed closely by P_3 (26.22 ± 27.82), and P_1 (25.16 ± 26.90). P_2 showed the lowest mean value (22.86 ± 24.44). The overlapping error bars indicate a high degree of variability among replicates, suggesting no significant differences across treatments. This interpretation is confirmed by the ANOVA results (p = 0.985), which demonstrate that

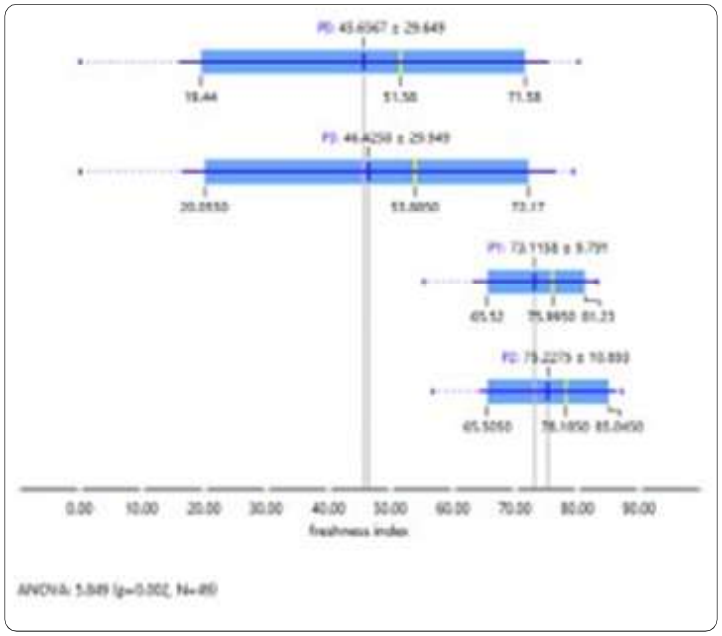

Fig. 5 : Effect of experimental treatments on freshness index of flowers

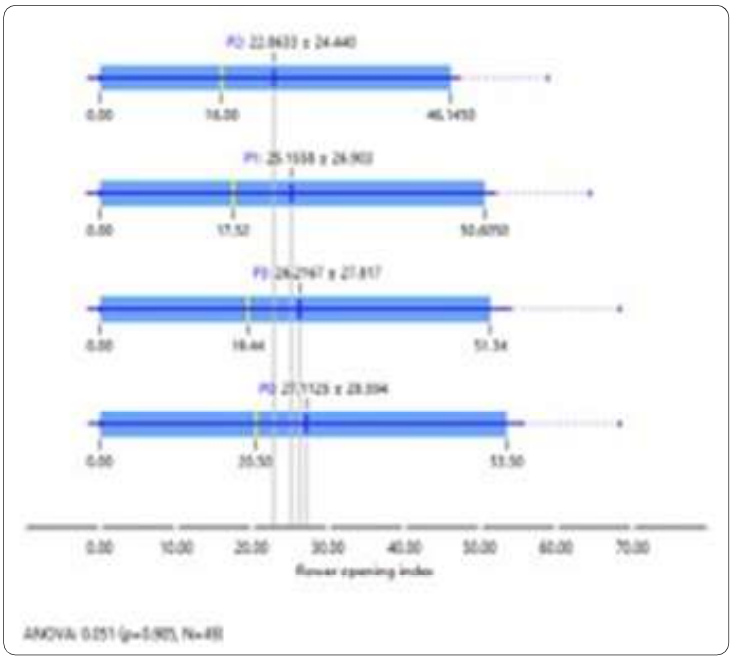

Fig. 6 : Effect of experimental treatments on flower opening index

the treatments did not significantly influence the flower opening index. The wide confidence intervals and close mean values suggest that external factors or natural variability may have played a greater role than the treatments themselves. Overall, the results emphasize the homogeneity of treatment effects on flower opening index in this experiment.

This study successfully demonstrated the effectiveness of proper packaging materials and the SVM algorithm in preserving the quality and predicting the shelf-life

of jasmine flowers. The study of different packaging methods showed that Corrugated Fiber board (CFB) with Polyethylene (PE) lining (P2) was the most effective in maintaining the jasmine flowers' freshness, with a maximum freshness index of 59.28 on the 16th day of storage. In contrast, traditional methods like bamboo baskets (P0) and nylon bags (P3) shows a significant and rapid decline in freshness. The research also highlighted that packaging is important for creating an optimum climate, regulating humidity and slowing down respiration to extend shelf life. Furthermore, the implementation of the SVM algorithm proved to be a powerful tool in predicting shelf-life of flower. The Polynomial kernel shows better performance over other kernels on both normalized and discretized datasets, achieving high correlation coefficients for both freshness index and flower opening index during training and testing. The SVM model's ability to minimize loss functions, as shown by the lowest RMSE values at a cost value of 166, confirms its accuracy in modelling and predicting the postharvest quality parameters of jasmine flowers. These results highlight the possibility of enhancing quality parameters of flower consistency and minimizing waste in the floral supply chain by integrating packaging material with predictive machine learning algorithms.

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Genetic Variability Studies in Ridge Gourd (*Luffa acutangula* (L.) Roxb.) for Yield and Yield Attributing Traits

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Abstract

The present investigation was conducted at the Department of Horticulture, UAS, GKVK, Bengaluru during *rabi* season of 2023. The experiment comprised of thirty-three genotypes and was laid out in Randomized Block Design with three replications. ANOVA revealed highly significant differences for all the growth, flowering and yield parameters. Among the genotypes evaluated, the best performing genotypes for growth parameters were IC-344512 with maximum vine length (4.31 m) and internodal length (11.48 cm), and Padmini with maximum number of primary branches (6.92) at 90 days after sowing. Similarly, Kerala Local-3 and IC-344652 were recorded to be the best-performing genotypes for earliness trait, days taken to first male flower (36.47 days) and female flower (39.22 days) appearance, respectively and IC-344652 was the earliest fruiting genotype (49.74 days). In case of yield and yield attributing parameters, Kolar Local-2 had the longest fruits (47.69 cm), IC-201145 had the maximum fruit diameter (5.35 cm) and Padmini had maximum average fruit weight (255.00 g). However, Kashi Khushi had maximum number of fruits (20.35) and maximum fruit set percentage (90.19 %). Significantly highest fruit yield per plant (3.90 kg) and fruit yield per hectare (20.27 t) was recorded in IC-344512.

Keywords : Genetic advance, Genetic variability, Heritability, Luffa acutangula, Ridge gourd

Ridge gourd (*Luffa acutangula* (L.) Roxb.) is a cucurbit belonging to the family Cucurbitaceae and the *Luffa* genus, characterized by a chromosome number of 2n = 26. While the genus *Luffa* includes seven species, only ridge gourd (*Luffa acutangula*) and sponge gourd (*Luffa cylindrica*, also known as *Luffa aegyptiaca*) are commonly grown for their edible fruits. Ridge gourd is popularly recognized by names such as *kalitori*, angled gourd, angled loofah and ribbed gourd. Its origin was in the subtropical regions of Asia, especially India, where wild forms are still found (Yadav *et al.,* 2015, Swarup, 2016).

The nutritional composition of ridge gourd fruit per 100 grams of the edible portion (after removal of the tough skin, which constitutes about 62% of the fruit) includes 94.2 grams of water, 70 kJ (17 kcal) of energy, 0.8 grams of protein, 0.1 grams of fat, 3.3 grams of carbohydrates, 1.7 grams of fiber, 12 mg of calcium, 32 mg of phosphorus, 0.3 mg of iron, 26 µg of carotene, 0.07 mg of thiamin, 0.02 mg of riboflavin, 0.4 mg of niacin, 37 µg of folate and 3 mg of ascorbic acid (Rathore *et al.,* 2017). Traditionally, ridge gourd has been utilized as a remedy for stomach disorders and fevers. It contains a gelatinous

substance known as 'luffein' which holds notable medicinal value and serves in identifying trypsin inhibitors. Moreover, juice extracted from fresh leaves is applied to children's eyes to manage granular conjunctivitis and to prevent eyelids from sticking during sleep due to excessive meibomian gland secretions (Suvedha *et al.,* 2024). The seeds possess purgative, emetic and anthelmintic properties, effects credited to the presence of the secondary metabolite cucurbitacin. Additionally, ridge gourd seeds have demonstrated antifungal, antitumor, antiviral effects, as well as inhibitory activity against HIV-1 integrase (Hou *et al.,* 2006).

The assessment and identification of superior genotypes within the existing germplasm are essential steps for the efficient utilization and enhancement of plant genetic resources in crop breeding programs (Pidigam *et al.,* 2019; Terfa and Gurnu, 2020). The success of genetic improvement in any crop depends largely on the extent of genetic diversity and the heritability of economically important traits (Saisupriya *et al.,* 2022). Greater genetic variability increases the likelihood of selecting superior genotypes. Genetic parameters such as genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) provide an indication of the level of variability within a genetic population. Estimates of genetic characteristics, including heritability and genetic advance, guide plant breeders in selecting elite genotypes from diverse genetic populations (Poornima *et al.,* 2023, Harshitha *et al.,* 2025). Keeping these factors in mind, a field study was conducted on ridge gourd to evaluate genetic variability, heritability and genetic advance related to yield and associated traits, with the aim of harnessing this variability for crop improvement.

Material and Methods

The research was conducted at the vegetable block of the Department of Horticulture, UAS, GKVK, Bengaluru, during *rabi* season of 2023. The experimental material consisted of 33 ridge gourd genotypes collected from various sources, and the list of ridge gourd genotypes is detailed in Table 1.

Table 1

List of ridge gourd genotypes used and their sources of collection

Germplasm	Source of collection
IC-344652	CoH, Bangalore
IC-335912	CoH, Bangalore
IC-124268	CoH, Bangalore
IC-144613	CoH, Bangalore
IC-92638	CoH, Bangalore
IC-201145	CoH, Bangalore
IC-126249	CoH, Bangalore
IC-344512	CoH, Bangalore
IC-344605	CoH, Bangalore
IC-146606	CoH, Bangalore
Green long	CoH, Bangalore
Thovinkere Local	CoH, Bangalore
Mysuru Local	Mysore
Kolar Local-1	Kolar
Kolar Local-2	Kolar
Padmini	Dharwad
Bangalore Local -1	Bangalore
Bangalore Local-2	Bangalore
Bagalkot Local-1	Bangalore
Bagalkot Local -2	Bagalkot
Bagalkot Local -3	Bagalkot
Kerala Local -1	Bagalkot
Kerala Local -2	Kerala
Kerala Local -3	Kerala
Jaipur Long	Kerala
Sannur Local	Sannur
Raichur Local -1	Raichur
Raichur Local-2	Raichur
Kashi Shivani	IIVR, Varanasi
Kashi Nanda	IIVR, Varanasi
Kashi Khushi	IIVR, Varanasi
Arka Sujat	IIHR, Bangalore
Arka Prasan	IIHR, Bangalore

Seeds were directly sown in the field following a randomized block design (RBD) with three replications, maintaining a planting distance of 1.0 × 1.5 meters. Farmyard manure was applied at the rate

of 25 tonnes per hectare, along with the recommended dose of fertilizer. Data were collected from five randomly selected plants within each plot. Observations were recorded for 21 parameters of ridge gourd. Variability for various quantitative traits was estimated using the analysis of variance procedure proposed by Panse and Sukhatme (1985). Genotypic and phenotypic coefficients of variation were computed according to Burton's formula (1952). Genetic advance was calculated based on the formula provided by Johnson *et al.* (1955), following the classification by Sivasubramanian and Menon (1973).

Results and Discussion

Mean Performance of Genotypes for Growth Parameters

Analysis of variance revealed significant variance observed among the genotypes studied for all the traits in this investigation.

Significant variation was observed among ridge gourd genotypes for vine length, internodal length, and number of primary branches (Table 2). IC-344512 exhibited the longest vine length (4.31 m), which was on par with Arka Prasan (4.27 m), Bangalore Local-1 (4.26 m), Raichur Local-2 (4.25 m) and IC-344652 (4.25 m). Kerala Local-2 displayed the shortest vine length (3.07 m).

Similarly, IC-344512 exhibited the maximum internodal length of 11.48 cm, followed by Arka Prasan (10.58 cm). whereas minimum internodal length (7.31 cm) was observed in Kerala Local-2.

In terms of the number of primary branches, Padmini exhibited maximum number of primary branches (6.92) on par with Bangalore Local-2 (6.86) and Bagalkot Local-3 (6.82). Conversely, IC-126249 exhibited the minimum number of primary branches (4.58).

The significant variation observed among the ridge gourd genotypes for vegetative traits like vine length, internodal length, and number of primary branches highlights the presence of substantial genetic diversity within the studied germplasm. This variability is fundamental for crop improvement, as it provides the basis for effective selection and breeding. Plant vigour, indicated by traits such as vine length, is essential for establishing a robust canopy that supports photosynthesis and subsequent fruit development and number of primary branches is another critical trait, as it directly influences the number of nodes available for flowering and fruit set, thereby impacting overall yield.

Mean Performance of Ridge Gourd Genotypes for Flowering Traits

Observations recorded for flowering traits are displayed in the Table 2. Significant variation was observed among 33 ridge gourd genotypes for days to first male and female flower appearance, node of first male and female flower appearance, days to 50 per cent flowering, and sex ratio. Kerala Local-3 exhibited the earliest first male flower appearance at 36.47 days, on par with IC-344652 at 37.34 days, whereas Kerala Local-2 exhibited the delayed male flowering at 45.25 days. Similarly, the earliest female flower appeared in IC-344652 at 39.22 days, which was on par with Kerala Local-3 (40.27 days) and Kashi Khushi (40.53 days), while IC-344605 exhibited delayed female flowering at 50.60 days.

Regarding node position, Kerala Local-2 and Bagalkot Local-2 had the highest nodes for the first male flower emergence, at 7.04 and 6.91, respectively. Conversely, Kerala Local-3 displayed the lowest node for first male flower appearance (3.27). For the node of first female flower appearance, Mysore Local exhibited the highest node for female flower appearance at 11.00, followed by IC-344605 (10.64), Raichur Local-1 (10.50), IC-144613 (10.48), Kerala Local-2 (10.46) and IC-92638 (10.41). Whereas, IC-344652 displayed lowest node for female flower appearance at 5.53.

In terms of flowering progress, IC-344652 exhibited early 50 per cent flowering at 44.12 days, statistically similar to Kerala Local-3 (45.18 days), IC-344512 (46.54 days) and Padmini (46.66 days), whereas IC-344605 displayed the delayed 50 per cent flowering at 56.62 days.

TABLE 2

Mean performance of ridge gourd genotypes for growth and flowering traits

Genotypes	Vine length (m)	Internodal length (cm)	Number of primary branches	DFMF	DFFF	NFMF	NFFF	DFF	SR
IC-344652	4.25	9.98	6.25	37.34	39.22	3.31	5.53	44.12	18.21
IC-335912	3.98	8.74	5.25	42.43	46.90	4.90	9.46	51.70	21.93
IC-124268	3.60	7.73	5.54	41.14	46.65	4.82	9.25	55.39	26.70
IC-144613	3.71	7.75	5.13	44.46	49.48	6.32	10.48	54.44	25.03
IC-92638	3.96	8.53	5.44	44.38	49.22	5.78	10.41	55.42	22.69
IC-201145	4.00	8.70	5.53	41.67	45.63	4.90	8.76	51.11	20.04
IC-126249	3.91	7.99	4.58	43.23	47.25	5.17	9.73	54.26	21.11
IC-344512	4.31	11.48	6.40	38.93	41.22	3.48	7.14	46.54	18.29
IC-344605	3.84	7.92	5.33	44.39	50.60	6.03	10.64	56.62	29.99
IC-146606	3.92	8.23	5.48	39.78	44.78	4.36	8.14	53.32	20.04
Green Long	3.17	7.65	5.06	43.04	47.93	5.07	9.88	53.98	24.30
Thovinkere Local	3.99	8.65	5.59	41.52	46.50	4.87	9.24	50.83	24.46
Mysore Local	3.16	7.53	5.86	44.63	50.42	4.50	11.00	56.03	27.47
Kolar Local-1	4.04	9.23	5.78	40.60	45.14	4.69	8.21	51.30	21.95
Kolar Local-2	4.18	9.68	6.31	39.43	42.30	3.94	7.40	47.59	18.24
Padmini	4.22	9.88	6.92	38.50	41.77	3.48	7.14	46.66	18.34
Bangalore Local-1	4.26	10.11	6.44	39.41	42.31	3.56	7.26	46.95	19.46
Bangalore Local-2	3.88	7.95	6.86	43.64	48.50	5.18	10.15	54.91	22.81
Bagalkot Local-1	4.00	8.95	5.74	40.61	45.57	4.72	8.55	52.17	24.33
Bagalkot Local-2	3.95	8.44	5.40	40.58	44.58	6.91	7.96	50.86	20.53
Bagalkot Local-3	4.02	9.14	6.82	42.44	48.08	4.94	9.99	54.77	27.13
Kerala Local-1	4.06	9.13	5.71	44.26	48.84	5.23	10.25	55.72	22.26
Kerala Local-2	3.07	7.31	5.16	45.25	49.42	7.04	10.46	54.65	21.49
Kerala Local-3	4.21	9.95	6.29	36.47	40.27	3.27	5.76	45.18	18.41
Jaipur Long	4.07	9.32	6.12	40.86	46.34	4.82	9.09	51.91	26.37
Sannur Local	3.25	7.83	5.66	40.38	45.51	4.40	8.49	52.39	24.60

Continued....

Table 2 Continued....

Genotypes	Vine length (m)	Internodal length (cm)	Number of primary branches	DFMF	DFFF	NFMF	NFFF	DFF	SR
Raichur Local-1	3.88	8.22	5.98	44.32	49.84	5.55	10.50	55.96	26.85
Raichur Local-2	4.25	9.99	6.19	39.44	42.82	4.00	7.60	48.88	18.35
Kashi Shivani	3.91	8.00	6.40	39.76	44.70	4.16	8.06	47.91	23.48
Kashi Nanda	3.85	8.11	6.52	44.44	49.03	6.09	10.26	54.98	22.38
Kashi Khushi	4.06	9.28	6.10	40.53	40.53	5.77	5.77	52.44	1.00
Arka Sujat	4.20	9.15	6.49	39.76	45.66	4.19	9.08	52.42	27.79
Arka Prasan	4.27	10.58	6.08	40.77	46.84	4.74	9.31	52.69	28.99
Mean	3.92	8.82	5.89	41.47	45.87	4.85	8.82	51.94	22.27
SE.m (±)	0.08	0.11	0.09	0.44	0.64	0.09	0.08	0.84	0.07
C.D. (5%)	0.23	0.30	0.22	1.23	1.82	0.25	0.23	2.36	0.19

DFMF- Days to first male flower appearance, DFFF-Days to first female flower appearance, NFMF-Node to first male flower appearance, NFFF- Node to first female flower appearance, DFF-Days to 50 per cent flowering, SR- Sex ratio (M:F)

The sex ratio varied significantly with the lowest sex ratio observed in IC-344652 (18.21) which was on par with Kolar Local-2 (18.24), IC-344512 (18.29), Padmini (18.34), Raichur Local-2 (18.35) and Kerala Local-3 (18.41), making these genotypes favourable for breeding due to their low male-to-female flower ratio. In contrast, IC-344605 exhibited the highest sex ratio at 29.99, while Kashi Khushi was unique in producing hermaphroditic flowers.

The significant variation observed in flowering traits among the 33 ridge gourd genotypes provides a strong foundation for breeding programs aimed at developing early-maturing and high-yielding varieties. Earliness, as indicated by days to first flower appearance and days to 50 per cent flowering, is a crucial trait for growers, as it can lead to higher market prices and allow for multiple cropping cycles or avoidance of late-season stresses. The wide range observed between the earliest and latest flowering genotypes under scores the substantial genetic variability available for manipulation through selection and hybridization. The node at which the first female flower appears is a critical indicator of earliness and yield potential. Genotypes that produce female flowers at lower nodes are highly valuable because they initiate fruit production earlier in their growth cycle, often leading to a greater number of fruits per plant. Conversely, genotypes which produce female flowers at higher nodes, tend to have a longer vegetative phase. This variation allows breeders to select parental lines to develop cultivars with specific plant architecture and maturity durations. In monoecious cucurbits like ridge gourd, the sex ratio is a key determinant of fruit yield. A lower ratio of male to female flowers is directly correlated with higher productivity. The genotypes having lower sex ratio are excellent for use as parents in breeding programs to develop progenies with enhanced fruit-setting ability.

Mean Performance of Ridge Gourd Genotypes for Fruiting Traits

Thirty-three ridge gourd genotypes exhibited significant variation across several important fruit traits studied (Table 3). Padmini exhibited early harvest at 49.74 days, on par with IC-344652 (50.25 days), IC-344512 (50.40 days) and Kerala Local-3

TABLE 3

Mean performance of ridge gourd genotypes for fruiting parameters

Genotypes	DFH	PFS	NR	FL	FD	TSS
IC-344652	50.25	78.25	10.03	28.28	4.45	3.98
IC-335912	57.86	61.75	9.07	36.05	5.01	4.52
IC-124268	57.66	53.77	10.07	27.19	4.68	4.13
IC-144613	60.82	53.61	10.10	22.54	3.52	4.21
IC-92638	59.35	59.39	10.03	23.92	4.01	3.94
IC-201145	57.35	65.44	9.33	27.75	5.35	3.88
IC-126249	57.88	60.82	10.13	22.25	5.05	4.06
IC-344512	50.40	74.34	10.87	32.42	4.30	4.50
IC-344605	61.90	52.79	10.53	23.40	5.19	3.92
IC-146606	56.95	67.46	10.64	27.20	4.82	4.20
Green Long	58.44	57.45	10.10	23.41	4.80	4.02
Thovinkere Local	56.85	53.67	10.07	22.97	4.38	4.63
Mysore Local	60.39	52.53	10.07	25.28	4.69	3.81
Kolar Local-1	55.82	61.13	10.53	23.40	5.16	3.81
Kolar Local-2	51.60	74.54	10.87	47.69	4.26	4.55
Padmini	49.74	75.60	10.07	29.71	4.52	4.03
Bangalore Local-1	52.70	72.56	10.60	27.69	4.47	4.41
Bangalore Local-2	57.24	58.69	10.03	24.14	4.60	4.41
Bagalkot Local-1	56.46	57.32	10.07	45.05	4.32	4.09
Bagalkot Local-2	56.56	61.13	10.07	31.76	4.30	4.22
Bagalkot Local-3	58.35	52.65	10.03	20.28	4.48	5.01
Kerala Local-1	59.74	60.28	10.07	44.15	3.79	4.90
Kerala Local-2	59.44	61.13	10.03	30.72	3.69	3.55
Kerala Local-3	50.77	76.13	10.07	46.41	4.01	3.69
Jaipur Long	56.42	52.15	10.07	32.06	3.75	4.06
Sannur Local	57.59	53.51	10.07	33.70	3.68	3.36
Raichur Local-1	60.30	54.39	10.07	40.55	3.97	3.38
Raichur Local-2	52.13	75.96	10.10	36.53	4.10	4.52
Kashi Shivani	55.26	57.72	10.07	25.46	4.67	4.18
Kashi Nanda	54.88	61.57	9.67	17.29	4.67	3.91
Kashi Khushi	55.24	90.19	9.87	13.59	4.17	3.72
Arka Sujat	54.93	63.88	10.07	33.14	4.19	4.34
Arka Prasan	55.20	63.66	10.00	45.16	4.03	4.30
Mean	56.26	62.89	10.09	30.03	4.40	4.13
SE.m (±)	0.57	0.48	0.20	0.58	0.05	0.09
C.D. (5%)	1.61	1.35	0.58	1.65	0.13	0.26

DFH- Days to first harvest, PFS- Percent fruit set (%), NR- Number of ridges per fruit, FL- Fruit length (cm), FD- Fruit diameter (cm), TSS-Total soluble solids (°B)

(50.77 days). In contrast, IC-344605 showed the late harvesting at 61.90 days.

Regarding fruit set percentage, highest fruit set percentage was observed in Kashi Khushi at 90.19 per cent, followed by IC-344652 (78.25%). Conversely Jaipur Long exhibited the lowest fruit set percentage (52.15%).

The highest number of ridges per fruit was observed in IC-344512 (10.87), followed by IC-146606 (10.64). The minimum number of ridges was noticed in IC-335912 (9.07).

Maximum fruit length was recorded in Kolar Local-2 (47.69 cm), which was statistically similar to Kerala Local-3 (46.41 cm), whereas minimum fruit length was noticed in Kashi Khushi (13.59 cm). Fruit diameter was maximum in IC-201145 (5.35 cm), followed by IC-344605 (5.19 cm) and Kolar Local-1 (5.16 cm). In contrast, IC-144613 exhibited the minimum fruit diameter at 3.52 cm.

Total soluble solids (TSS) content was highest in Bagalkot Local-3 (5.01 °B), which was statistically similar to Kerala Local-1 (4.90 °B), whereas Kashi Khushi exhibited the lowest Total Soluble Solids (3.72 °B).

The significant variation observed among the 33 ridge gourd genotypes for critical fruit traits highlights the rich genetic diversity available for breeding. This diversity is essential for developing improved cultivars that meet the demands of both growers and consumers, focusing on earliness, yield and quality.

This study successfully identified genotypes with superior individual and combined traits. A strategic breeding program could involve crossing genotypes to combine desirable traits. For instance, hybridizing a high-yielding genotype with a long-fruited, high-TSS genotype could lead to the development of superior hybrids that integrate high yield with desirable market and quality characteristics.

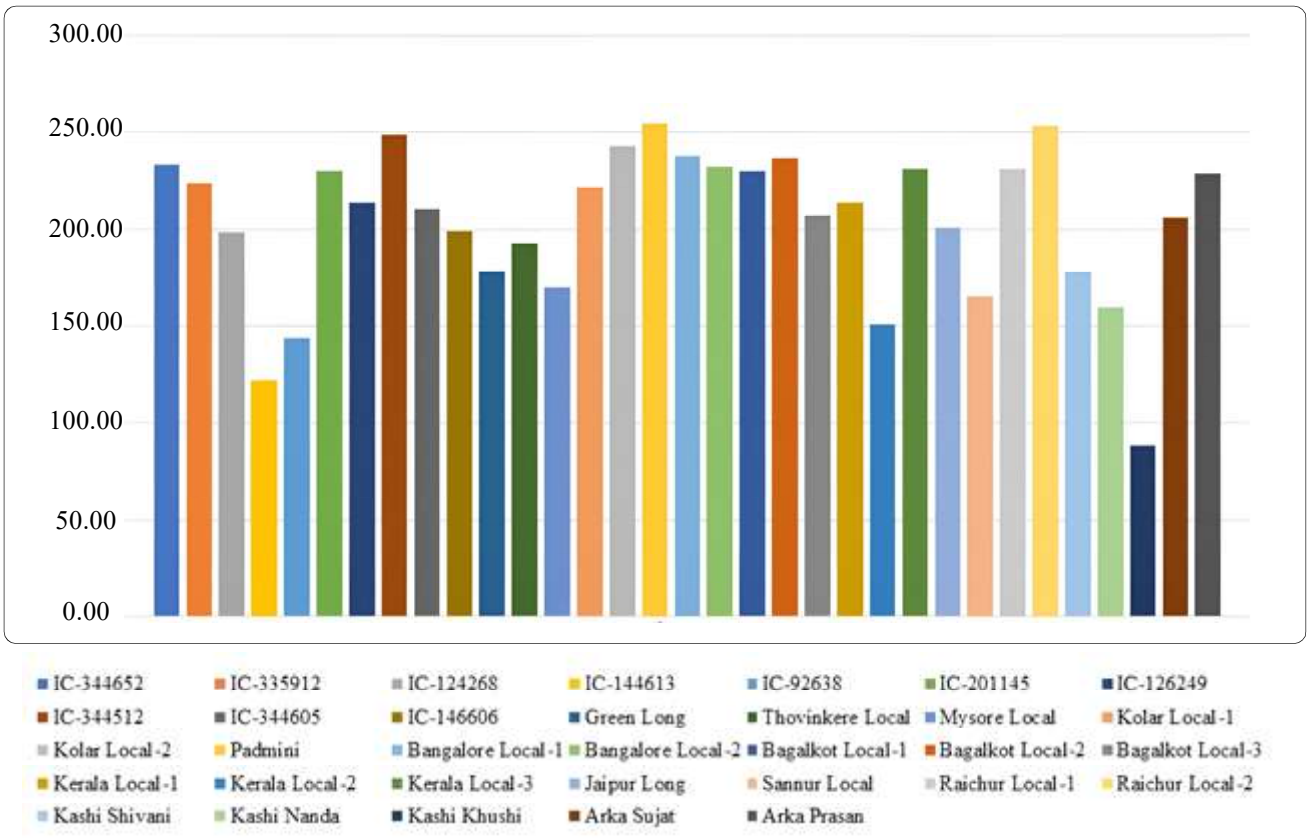

Fig. 1 : Mean performance of ridge gourd genotypes for fruit weight (g)

TABLE 4

Mean performance of ridge gourd genotypes for yield and yield attributing parameters

Genotypes	NFV	YPV	YPH	NSF	100 SW
IC-344652	16.30	3.80	19.78	90.63	14.19
IC-335912	10.85	2.43	12.64	105.63	8.29
IC-124268	8.82	1.75	9.12	118.97	15.23
IC-144613	8.85	1.14	6.85	98.77	13.29
IC-92638	10.04	1.45	7.50	93.61	12.17
IC-201145	11.96	2.75	14.30	198.67	15.28
IC-126249	10.93	2.34	12.15	131.90	16.19
IC-344512	16.06	3.90	20.27	115.80	14.24
IC-344605	7.63	1.61	8.35	211.23	13.12
IC-146606	12.60	2.51	13.06	136.29	16.22
Green Long	9.55	1.70	8.86	98.13	12.24
Thovinkere Local	8.89	1.71	8.91	96.00	12.31
Mysore Local	8.12	1.38	7.18	102.27	15.14
Kolar Local-1	10.67	2.37	12.31	114.40	15.22
Kolar Local-2	15.87	3.86	20.06	72.27	14.29
Padmini	14.86	3.79	19.70	143.30	10.30
Bangalore Local-1	13.98	3.33	17.30	188.07	15.16
Bangalore Local-2	9.88	2.29	11.93	62.13	15.17
Bagalkot Local-1	9.52	2.19	11.39	144.51	17.29
Bagalkot Local-2	11.12	2.63	13.69	118.63	12.33
Bagalkot Local-3	8.28	1.71	8.90	188.70	16.22
Kerala Local-1	10.51	2.25	11.68	189.53	17.22
Kerala Local-2	10.92	1.65	8.57	128.68	12.36
Kerala Local-3	14.84	3.43	17.86	93.60	16.17
Jaipur Long	8.36	1.68	8.72	114.23	14.24
Sannur Local	8.85	1.46	7.60	113.30	13.24
Raichur Local-1	8.73	2.02	10.49	131.17	16.21
Raichur Local-2	15.54	3.85	20.18	189.73	13.35
Kashi Shivani	9.63	1.72	8.90	104.33	17.20
Kashi Nanda	10.50	1.67	8.69	100.80	14.21
Kashi Khushi	20.35	1.79	9.29	41.27	9.19
Arka Sujat	9.82	2.02	10.51	98.27	15.28
Arka Prasan	9.74	2.23	11.59	125.22	15.14
Mean	11.29	2.32	12.07	123.03	14.17
SE.m (±)	0.08	0.02	0.09	0.30	0.08
C.D. (5%)	0.23	0.05	0.24	0.86	0.24

NFV - Number of fruits per vine, YPV - Yield per vine (Kg), YPH - Yield per hectare (t),
NSF - Number of seeds per fruit, SW- Seed weight (g)

Mean Performance of Ridge Gourd Genotypes for Yield and Yield Attributing Traits

Observation for yield and yield contributing traits are presented in the Table 4. Kashi Khushi produced the highest number of fruits per vine at 20.35, followed by IC-344652 (16.30) and IC-344512 (16.06), whereas IC-344605 exhibited the minimum number of fruits per vine (7.63).

The data regarding average fruit weight was presented in Fig.1. The genotype Padmini exhibited the maximum average fruit weight (255.00 g), on par with Raichur Local-2 (253.67 g) and IC-344512 (248.60 g). Conversely, minimum average fruit weight was recorded in Kashi Khushi (87.80 g).

Significant difference was observed for yield (Table 4). Yield per vine was maximum in IC-344512 (3.90 kg), which was statistically similar to Kolar Local-2 (3.86 kg), whereas IC-144613 produced the minimum yield per vine (1.14 kg). Similarly, yield per hectare was maximum in IC-344512 (20.27 t), which was statistically similar to Raichur Local-2 (20.18 t) and Kolar Local-2 (20.06 t). Conversely IC-144613 exhibited the lowest yield per hectare (6.85 t).

Number of seeds per fruit varied significantly among genotypes. IC-344605 exhibited the maximum number of seeds per fruit (211.23), followed by IC-201145 (198.67), whereas minimum number of seeds per fruit was recorded in Kashi Khushi (41.27). The 100 seeds weight ranged from the maximum in Bagalkot Local-1 (17.29 g) and Kerala Local-1 (17.22 g) to the minimum in IC-335912 (8.29 g).

The significant variation was observed in yield and yield contributing traits among the ridge gourd genotypes which is important for breeding of high-yielding cultivars. Yield is a complex quantitative trait that results from the interplay of several components, primarily the number of fruits per vine and the average fruit weight. This study effectively demonstrated the genetic variability present for these components and identified genotypes with superior performance.

The identification of superior genotypes for specific traits provides a clear path for future breeding efforts. A strategic hybridization program could involve crossing genotypes with complementary traits. For instance, crossing Kashi Khushi (for its high fruit number and low seed count) with a genotype like Padmini (for high average fruit weight) could potentially break the negative correlation between these traits and produce transgressive segregants with both high fruit numbers and desirable weight.

Genotypic and Phenotypic coefficient of Variability

The phenotypic coefficient of variation (PCV) captures the total observable diversity in a trait-accounting for both genetic and environmental influences. This measure reflects the complete spectrum of variation seen in phenotypes, which can fluctuate based on factors like cultivation methods, soil type and climatic conditions. A higher PCV suggests that environmental factors play a greater role in shaping the traits expression. In contrast, the genotypic coefficient of variation (GCV) isolates the heritable portion of this variation. By excluding environmental effects, the GCV reveals the extent to which differences among individuals are due solely to genetic factors. This distinction is valuable for selecting superior genotypes and for predicting the potential success of breeding programs, as it highlights the component of variability that can be passed on to future generations. GCV is often lower than PCV because it isolates genetic contributions. Both GCV and PCV were categorized based on their magnitude of variation as low (less than 10%), moderate (10 to 20%) and high (more than 20%).

The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for various traits are presented in Table 5. For all the traits assessed, PCV values consistently exceeded GCV values, indicating that phenotypic variation results from both genetic and environmental influences. Yield per vine exhibited the highest variation, with GCV 35.81 per cent and PCV at 35.83 per cent, followed by yield per hectare at GCV = 35.50 per cent and

TABLE 5

Variability parameters for yield and yield contributing characters among 33 genotypes of ridge gourd

Characters	Range		Mean	GV	PV	GCV (%)	PCV (%)	h^2_{bs} (%)	GA	GA
	Min	Max								
Germination (%)	65.93	90.97	84.35	54.59	55.80	8.76	8.86	97.83	15.05	17.85
Vine length (m)	3.16	4.31	3.92	0.10	0.12	8.18	8.94	83.51	0.60	15.39
Internodal length (cm)	7.31	11.48	8.82	0.98	1.01	11.21	11.40	96.76	2.01	22.73
Primary branches	4.58	6.92	5.89	0.32	0.34	9.61	9.90	94.48	1.14	19.26
Days to first male flower appearance	36.47	45.25	41.47	5.16	5.73	5.48	5.77	90.06	4.44	10.70
Days to first female flower appearance	39.22	50.60	45.87	9.78	10.32	6.73	7.16	88.45	5.98	13.04
Node of first male flower appearance	3.27	7.04	4.85	0.93	0.96	19.90	20.15	97.56	1.97	40.49
Node of first female flower appearance	5.53	11.00	8.82	2.27	2.29	17.07	17.14	99.17	3.09	35.02
Days 50% Flowering	44.12	56.62	51.94	11.73	13.31	6.43	7.03	83.71	6.29	12.12
Sex ratio	1	29.99	22.27	26.27	26.29	23.01	23.02	99.95	10.56	47.39
Days to first harvest	50.25	61.90	56.26	10.32	11.30	5.71	5.97	91.33	6.32	11.24
Number of fruits per vine	7.63	20.35	11.29	9.19	9.21	26.85	26.88	99.79	6.24	55.25
Fruit set (%)	52.51	90.04	62.89	88.98	89.67	15.00	15.06	99.23	19.36	30.78
Number of ridges	9.07	10.87	10.09	0.07	0.19	2.61	4.36	35.84	0.32	3.22
Fruit length (cm)	13.59	47.69	30.03	72.46	73.47	28.05	28.25	98.62	17.41	57.38
Fruit diameter (cm)	3.52	5.35	4.40	0.22	0.23	10.65	10.80	97.33	0.95	21.65
Average fruit weight (g)	87.80	255.00	204.11	1556.82	1557.37	19.33	19.34	99.97	81.27	39.82
TSS(°B)	3.36	5.01	4.13	0.14	0.17	9.12	9.89	85.26	0.72	17.36
Number of seeds per fruit	41.27	211.23	123.03	1636.19	1636.46	32.88	32.88	99.98	83.32	67.72
100 Seed weight (g)	8.29	17.29	14.17	4.77	4.79	15.41	15.44	99.57	4.49	31.68
Yield per vine (kg)	1.14	3.90	2.32	0.69	0.69	35.81	35.83	99.87	1.71	73.71
Yield per hectare (t)	6.85	20.27	12.07	18.37	18.39	35.50	35.53	99.88	8.82	73.09

GV-Genotypic variance, PV-Phenotypic variance, GCV-genotypic coefficient variation, PCV-Phenotypic coefficient of variation, h^2_{bs}- Heritability, GA-Genetic advance, GAM-Genetic advance as percent of mean

PCV = 35.53 per cent, respectively. Conversely, the number of ridges showed the lowest variability, with GCV and PCV of 2.61 per cent and 4.36 per cent, respectively. Traits such as sex ratio (GCV=23.01% PCV=23.02%), number of fruits per vine (GCV=26.85 %, PCV=26.88%), fruit length (GCV=28.05%, PCV=28.25%) and number of seeds per fruit (GCV=32.88%, PCV=32.88%) demonstrated high genetic variability. High variability has been reported by earlier researchers in bottle gourd (Rambabu *et al.*, 2017) and in bitter gourd (Talukder *et al.*, 2018). Moderate variation was observed for internodal length (GCV=11.21%, PCV=11.40%), node of first male flower appearance (GCV=19.90%, PCV=20.15%), node of first female flower appearance (GCV=17.07%, PCV=17.14%), fruit set percentage (GCV=15.00%, PCV=15.06%), fruit diameter (GCV=10.65%, PCV=10.80%), average fruit weight (GCV=19.33%, PCV=19.34%), and 100-seed weight (GCV=15.41%, PCV=15.44 %). Low variability was recorded for germination percentage (GCV=8.76%, PCV=8.86%), vine length (GCV=8.18%, PCV=8.94%), number of primary branches (GCV=9.61%, PCV=9.90%), days to first male flower appearance (GCV=5.48%, PCV=5.77%), days to first female flower appearance (GCV=6.73 %, PCV=7.16%), days to 50 per cent flowering (GCV=6.43%, PCV7.03%), days to first harvest (GCV=5.71%, PCV=5.97%) and total soluble solids (GCV=9.12%, PCV=9.89%).

The analysis of the Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) provides a quantitative measure of the genetic and observable variability present in the ridge gourd germplasm. The consistent observation that PCV values were higher than GCV values for all traits is expected, as it confirms that the total phenotypic expression is a composite of genetic effects and environmental influences. However, the narrow gap between the PCV and GCV values for most traits, indicates that the environmental influence was relatively small, suggesting that the observed variation is largely attributable to genetic factors.

Heritability and Genetic Advance

In a broad sense, heritability is the ratio of genetic variance to phenotypic variance, a measure of how effectively selection succeeds. Identifying superior genotypes with diverse genetic pools as one of its advantages. Selecting superior genotypes based on their quantitative trait indicators was made easier by high heritability scales. A high heritability estimate suggests that there are many fixed additive genes present, which enables target selection to improve traits. Combining advances and heritability is an essential prerequisite for efficient selection. For predicting the outcomes of finding high performing genotypes. It was categorized in three levels *viz*., High heritability (more than 60%), moderate (30 to 60%) and low (less than 30%). Likewise genetic advance also classified into three categories, high genetic advance (more than 20%), moderate (10 to 20%) and low (less than 10%).

Table 5, presents the heritability and genetic advance estimates for the traits studied, revealing that all parameters exhibited high heritability values. Traits demonstrating both high heritability and high genetic advance as per cent of mean included vine length (96.76% heritability, 22.73% genetic advance as per cent of mean), node of first male flower appearance (97.56%, 40.49%), node of first female flower appearance (99.17%, 35.02%), sex ratio (99.95%, 47.39%), number of fruits per vine (99.79%, 55.25%), fruit set percentage (99.23%, 30.78%), fruit length (98.62%, 57.38%), fruit diameter (97.33%, 21.65%), average fruit weight (99.97%, 39.82%), number of seeds per fruit (99.98%, 67.72%), 100-seed weight (99.57%, 31.68%), yield per vine (99.87%, 73.71%) and yield per hectare (99.88%, 73.09%).

The analysis of heritability and genetic advance provides crucial insights for designing an effective breeding strategy, indicating the extent to which a trait is genetically controlled and the potential for improvement through selection. The finding that all studied traits exhibited high heritability is a significant result, suggesting that the observed phenotypic

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variation is predominantly due to genetic factors rather than environmental influences. This high degree of genetic control makes the selection process more reliable and predictable. The combination of high heritability with high genetic advance as a per cent of mean is particularly powerful. This pairing, observed for most of the economically important traits, indicates the predominance of additive gene action in their inheritance. Traits governed by additive gene effects are highly responsive to selection, as the phenotype of an individual is a good indicator of its breeding value. Consequently, simple selection methods, such as mass selection or progeny selection, are expected to be highly effective in achieving significant genetic improvement in a relatively short period. These results align with the findings of Harshitha *et al.* (2019 and Chinthala pudi *et al.* (2021) in ridge gourd.

The study revealed considerable variability in several important traits, including sex ratio, number of fruits per vine, fruit length, number of seeds per fruit, yield per vine and yield per hectare. Moderate variability was observed for traits such as internodal length, the node of first male and female flower appearance, fruit set, fruit diameter and average fruit weight. Traits exhibiting both high heritability and high genetic advance include vine length, sex ratio, number of fruits per vine, fruit length, fruit diameter, average fruit weight, yield per plant and yield per hectare. The combination of high heritability and significant genetic advance indicates that these traits are predominantly controlled by additive gene effects. This genetic architecture allows for effective selection and improvement of these traits in future breeding generations using the genotypes evaluated in this study.

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Performance of Tomato (*Solanum lycopersicum* L.) Under Urban Horticulture as Influenced by Organo-Mineral Fertilizers

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Abstract

An experimental trial was undertaken to evaluate the efficacy of organo-mineral fertilizers on growth and yield of tomato grown under urban horticulture. Two different organo-mineral fertilizer formulations *i.e.,* Potting Mixtures and Nutrient Supplement cakes were tested in this study. The farm yard manure or potting mixture was used as growing medium and nutrient supplements cakes were applied at 100 and 75 per cent of the recommended dose of phosphorus. The experiment included three factors with two levels each resulting in eight treatments, replicated thrice, to assess their influence on tomato growth and yield. The results demonstrated that the application of organo-mineral fertilizers significantly improved tomato plant performance. The treatment which received Soil + OMF + 1-% RDP through NS1 cake recorded the highest enhancement in growth parameters. In terms of yield attributes also, the same treatment showed superior outcomes with increased number of clusters per plant (8.78), number of flowers per cluster (5.67), number of fruits per plant (31.67), fruit diameter (3.25 cm), fruit weight (93.45 g) and fruit yield (2.97 kg plant^{-1}).

Keywords : Urban horticulture, Performance of tomato, Growth, Yield, Potting mixture, Nutrient supplements

Urban horticulture has emerged as a viable approach to ensure sustainable food supply and nutritional security for urban populations. It involves the cultivation of fruits, vegetables, aromatic and ornamental plants suitable for urban environments (Eigenbrod & Gruda, 2015).

Crop like tomato thrives in small spaces and containers. Tomato grow vertically on stakes. However, urban horticulture often faces challenges related to water and nutrient management. Frequent watering, poor substrate fertility and unbalanced fertilization practices lead to leaching, nutrient deficiencies and reduced plant growth (Owen *et al.,* 2018).

Tomato (*Lycopersicon esculentum* L.), a major crop after potato, is widely consumed for its unique taste and high vitamin C content. It is rich in antioxidants like lycopene, along with vitamins A, C, E, K, potassium, folate and fibre, it offers significant nutritional benefits. However, its productivity is limited by pests, diseases, moisture stress, temperature extremes and improper fertilizer use (Frusciante *et al.,* 2017).

Organo-mineral fertilizers (OMFs) offer a sustainable solution by combining organic and mineral nutrient sources. They provide controlled nutrient release, improve soil health and enhance plant growth. OMFs improve physical properties such as aggregate

stability, aeration and water retention, as well as chemical attributes like pH, CEC and nutrient availability. Biologically, OMFs stimulate microbial diversity and activity by supplying balanced nutrients for soil microbes (Barbosa *et al.,* 2024).

The organic component of OMFs such as compost, biochar and peat possesses functional groups and porous structures that retain nutrients, reduce leaching and support gradual nutrient release. This slow-release mechanism improves nutrient use efficiency, reduces fertilizer loss and enhances crop quality and yield (Saha *et al.,* 2018). Hence, this study was undertaken to formulate two OMF types potting mixtures, nutrient supplement cakes as low-cost alternatives to commercial fertilizers. The objective was to evaluate their efficiency in urban horticulture using tomato as test crop.

Material and Methods

The organo-mineral fertilizers were prepared by blending organic manure with mineral fertilizers in definite proportions to obtain potting mixture (PM) designed to serve as a growing medium for urban horticulture crops and nutrient supplements (NS) suitable for horticultural crops like tomato, french bean and radishcrops. The experiment was conducted over the course of 120 days, during *kharif* 2024.

Preparation of Organo-Mineral Fertilizers

The synthesis of organo-mineral fertilizers, intended as PM, was meticulously executed through the strategic amalgamation of organic and mineral sources. The organic component, specifically compost, served as a carbonaceous base, while the mineral inputs encompassed phosphorus and salts of nitrogen, potassium and a spectrum of micronutrients.

To formulate organo-mineral fertilizer a well-decomposed compost was collected from field unit, College of Agriculture, Mandya as the organic base and mineral nutrients such as rock phosphate a slow-release phosphorus source, potassium nitrate as a source of both potassium and nitrogen, sulphate salts of essential micro nutrients such as $ZnSO_4$, $FeSO_4$, $MnSO_4$ and $CuSO_4$ were utilized.

OMF-PM was prepared by mixing compost with rock phosphate in different ratios of 1:1, 2:1, 5:1 and 10:1 to test as a growing medium for urban horticulture crops. A definite proportion of potassium nitrate and micronutrient sulphates at lower concentration were added uniformly to all mixtures to maintain uniformity of other nutrients. This mixing process culminated in the formulation of four distinct PM variants, designated as PM1, PM2, PM3 and PM4. Each combination was systematically engineered to exhibit unique nutrient profiles and physicochemical attributes, catering to diverse urban horticulture requisites.

The synthesis of organo-mineral fertilizers, intended as NS was formulated using four specific compost-to-rock phosphate ratios: 1:1, 1:2, 1:5 and 1:10. To ensure uniformity in nutrient content across all samples, definite amount of potassium nitrate and micronutrient sulphates (iron, manganese, copper and zinc) were added to each mixture to obtain high grade nutrient matrix. These elements were meticulously combined with ancillary substances such as binding agent gum acacia to enhance the physical and functional integrity of the resultant formulations.

The resultant organo-mineral paste was employed in the production of cakes *via* the caking process. This procedure involved the utilization of rectangular moulds to shape the paste into compact, solid forms. Upon moulding, the resultant cakes were subjected to an standard drying protocol at 65 °C, the moisture removal strategy. The caking process is used to prepare four formulations, namely NSC1, NSC2, NSC3 and NSC4, each distinguished by its nutrient profile and structural attributes.

Evaluation of the Efficacy of Prepared Organo-Mineral Fertilizers on Growth and Yield of Tomato

Based on the results obtained from the incubation studies, among the four distinct OMF-Pottimg mixtures, OMF-PM4 was choosen as best in terms

of nutrient release pattern and designated as OMF-PM and among the four distinct OMF-NSC formulated, OMF-NS3 Cake and OMF-NS4 Cake choosen as best and designated as OMF-NS1 and OMF-NS2 cake, respectively. The selected PM and NSC were used to evaluate their efficacy for tomato crop in growbags.

Surface soil sample collected from College of Agriculture, V.C. Farm, Mandya was used to fill the grow bags. Large lumps were crushed and roots and under composed litter was removed and used for filling the grow bags.

Grow bags measuring 12 × 12 inches and capable of holding up to twenty kilograms were employed in the study. Fifteen kilogram of soil was taken on a clean plastic sheet to which calculated quantity of FYM/OMF-PM was added (2 parts of soil: 1 part of FYM/OMF-PM) treatment wise and mixed well with the soil and was filled to each grow bag.

Based on the recommended phosphorus equivalent of the crop, OMF-NS cakes were applied treatment wise, remaining nitrogen and potassium were supplied through chemical fertilizers. Nitrogen was applied through urea and potassium was applied using muriate of potash (MOP) as per calculation. Split application of nitrogen fertilizer was given half as basal dose and other half after respective requirement of crop.

Tomato (*Lycopersicum esculentum* L.) was utilized as the test crop; the variety used here is 'Arka Samrat'. The suggested fertilizer dosage is 250:250:250 N, P_2O_5, K_2O. To meet out the phosphorus requirement 50 g of cake was applied. The duration of the crop was 115-120 days and there were eight treatments. The experiment included three factors with two levels each resulting in eight treatments, replicated thrice. The design choosen was Factorial Completely Randomized Design (FCRD). Timely required cultural practices like weeding and watering were done as crop maintenance. At 30, 60, 90 and at 120 DAT, morphological observations such as plant height (cm), number of leaves, number of branches per plant, internodal length, chlorophyll content, shoot and fruit dry weight (g plant^{-1}) were noted. Based on the yield from each plant, the amount of fruit was estimated and expressed as kg plant^{-1}. Following the suggested set of practices allowed the crop to be maintained. The data was statistically analysed using Gomez and Gomez's (1984) methodology. The software used for data analysis is two way ANOVA.

Results and Discussion

Effect of Organo-Mineral Fertilizers on Growth and Yield of Tomato

Growth Parameters of Tomato

Plant Height (cm)

The influence of organo-mineral fertilizers on plant height of tomato at different growth stages are presented in Fig. 1. Combined application of potting mixtures and nutrient supplements at two different levels affected plant height significantly. Application of Soil + OMF-PM recorded significantly highest plant height of 64.35, 90.98, 117.09 and 127.20 cm at 30, 60, 90 and 120 DAT, respectively, than compared to Soil+ FYM (56.47, 82.07, 105.08 and 116.66 cm, respectively). Among the two nutrient supplements, OMF-NS1 Cake recorded highest plant height of 61.92, 88.55, 113.91 and 124.77 cm, respectively, than OMF-NS2 Cake (58.90, 84.50, 108.26 and 119.09 cm, respectively). Recommended dose of P at 100 per cent recorded highest plant height of 63.58, 92.70, 117.07 and 128.92 cm, respectively, than 75 per cent equivalent RDP level (57.25, 80.34, 105.10 and 114.93 cm, respectively).

Among the different treatment combinations, application of OMF-PM + 100 per cent equivalent RDP through OMF-NS1 Cake recorded maximum plant height (70.23 cm, 99.35 cm, 125.47 cm and 135.58 cm, respectively) at 30, 60, 90 and 120 DAT crop and was found to be on par (65.72 cm, 94.84 cm, 120.96 cm and 131.07 cm, respectively) with treatment which received OMF-PM + 100 per cent equivalent RDP through OMF-NS2 Cake and minimum (50.43 cm, 70.44 cm, 91.12 cm and 100.12 cm, respectively) was recorded in Soil + FYM + 75 per cent equivalent RDP through OMF-NS2 Cake.

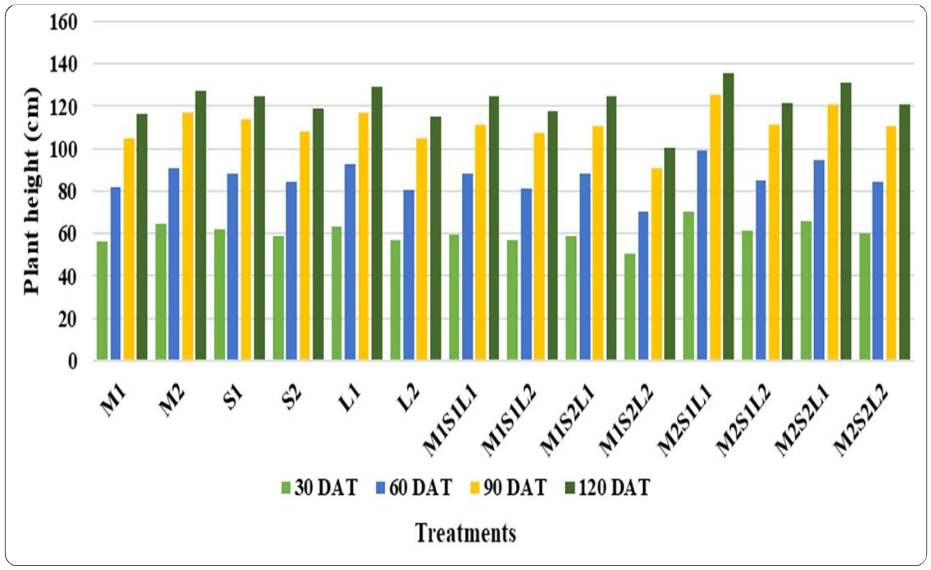

Fig. 1 : Effect of organo-mineral fertilizers on plant height of tomato

Treatment details :

Factor M : Potting mixtures	Factor S : Nutrient supplements	Factor L : Levels
M1 : Soil + FYM	S1 : OMF-NS1 Cake	L1 : 100% equivalent RDP
M2 : Soil + OMF-PM	S2 : OMF-NS2 Cake	L2 : 75% equivalent RDP

TABLE 1

Treatment details

Factor 1 : *Potting mixture*

M1 - Soil + FYM

M2 - Soil + OMF-PM

Factor 2 : Nutrient supplements

S1 - OMF-NS1 Cake

S2 - OMF-NS2 Cake

Factor 3 : Levels

L1 - 100% equivalent RDP

L2 - 75% equivalent RDP

Interactions

M1S1L1	Soil + FYM + 100% equivalent RDP through OMF-NS1 Cake
M1S1L2	Soil + FYM + 75% equivalent RDP through OMF-NS1 Cake
M1S2L1	Soil + FYM + 100% equivalent RDP through OMF-NS2 Cake
M1S2L2	Soil + FYM + 75% equivalent RDP through OMF-NS2 Cake
M2S1L1	Soil + OMF-PM + 100% equivalent RDP through OMF-NS1 Cake
M2S1L2	Soil + OMF-PM + 75% equivalent RDP through OMF-NS1 Cake
M2S2L1	Soil + OMF-PM + 100% equivalent RDP through OMF-NS2 Cake
M2S2L2	Soil + OMF-PM + 75% equivalent RDP through OMF-NS2 Cake

Note : OMF-Organo-mineral fertilizer; PM-Potting mixture; NS- Nutrient supplement; RDP- Recommended dose of phosphorus

Number of Branches Per Plant

The number of branches per plant differed significantly with the application organo-mineral fertilizers at all growth stages (Table 2). Significantly higher number of branches per plant at 30, 60, 90 and 120 DAT of 10.48, 12.57, 14.67 and 15.27, respectively was recorded with Soil + OMF-PM and the relatively lowest number of branches per planner was recorded with Soil + FYM. The mean values of number of branches varied significantly by application of nutrient supplements at different levels, however higher number of branches were recorded with the OMF-NS1 Cake (9.52, 11.52, 13.53 and 14.11, respectively) as compared to OMF-NS2 Cake. Among the levels, 100 per cent equivalent RDP significantly higher number of branches *viz.,* 11.12, 13.29, 15.52 and 15.75, respectively as compared to the 75 per cent equivalent RDP.

Higher number of branches per plant (11.76, 14.01, 16.26 and 16.50, respectively) was recorded in Soil + OMF-PM + 100 per cent equivalent RDP through OMF-NS1 Cake at 30, 60, 90 and 120 DAT which was statistically at par with OMF-PM + 100 per cent equivalent RDP through OMF-NS2 Cake, (11.29, 13.46, 15.71 and 15.95, respectively) treatment. The lower number of branches (6.90, 8.53, 10.15 and 11.01, respectively) was recorded in Soil + FYM + 75 per cent equivalent RDP through OMF-NS2 Cake at all the stages.

Number of Leaves Per Plant

Data in Table 3 indicates that number of leaves per plant differed significantly at different growth stages of tomato crop due to application of organo-mineral fertilizers. Among potting mixtures, at 30, 60, 90 and 120 DAT, significantly higher number of leaves per plant was recorded in Soil + OMF-PM (16.90, 66.12, 79.28 and 97.06, respectively). Whereas among nutrient supplements, OMF-NS1 Cake recorded significantly higher number of leaves per plant *i.e.,* 16.46, 64.63, 77.56 and 94.96, respectively. Among levels, 100 per cent equivalent RDP level recorded significantly higher number of leaves per plant *viz.,* 17.23, 67.85, 81.52 and 99.87, respectively.

Table 2

Effect of organo-mineral fertilizers on number of branches at different growth stages of tomato

Treatment	Number of branches			
	30 DAT	60 DAT	90 DAT	120 DAT
Potting mixtures				
M1	9.25	11.21	13.21	13.77
M2	10.48	12.57	14.67	15.27
S. Em±	0.10	0.12	0.14	0.15
CD@1%	0.42	0.50	0.58	0.60
Nutrient supplements				
S1	10.22	12.26	14.34	14.93
S2	9.52	11.52	13.54	14.11
S. Em±	0.10	0.12	0.14	0.15
CD@1%	0.42	0.50	0.58	0.60
Levels				
L1	11.12	13.29	15.52	15.75
L2	8.61	10.49	12.36	13.29
S. Em±	0.10	0.12	0.14	0.15
CD@1%	0.42	0.50	0.58	0.60
MXS	NS	NS	NS	NS
SXL	NS	NS	NS	NS
MXL	NS	NS	NS	NS
MXSXL				
M1S1L1	10.90	12.90	15.07	15.28
M1S1L2	8.67	10.63	12.58	13.54
M1S2L1	10.54	12.79	15.04	15.25
M1S2L2	6.90	8.53	10.15	11.01
M2S1L1	11.76	14.01	16.26	16.50
M2S1L2	9.53	11.49	13.44	14.40
M2S2L1	11.29	13.46	15.71	15.95
M2S2L2	9.34	11.30	13.25	14.21
S. Em ±	0.20	0.24	0.28	0.29
CD @ 1 %	0.83	1.00	1.17	1.21

Factor M : Potting mixturesFactor; S : Nutrient supplements
Factor L : Levels
M1 : Soil + FYMS1: OMF-NS1 CakeL1 : 100% equivalent RDP
M2 : Soil + OMF-PMS2: OMF-NS2 CakeL2 : 75% equivalent RDP

Note : OMF-PM- 10C:1RP, OMF-NS1 cake-1:2.75:1 and OMF-NS2 cake-1:2.5:1

Table 3

Effect of organo-mineral fertilizers on number of leaves at different growth stages of tomato

Treatment	Number of branches			
	30 DAT	60 DAT	90 DAT	120 DAT
Potting mixtures				
M1	15.11	59.52	71.69	87.54
M2	16.90	66.12	79.28	97.06
S. Em ±	0.16	0.63	0.76	0.93
CD @ 1%	0.67	2.61	3.14	3.84
Nutrient supplements				
S1	16.46	64.63	77.56	94.96
S2	15.54	61.00	73.41	89.64
S. Em ±	0.16	0.63	0.76	0.93
CD @ 1%	0.67	2.61	3.14	3.84
Levels				
L1	17.23	67.85	81.52	99.87
L2	14.78	57.78	69.45	84.73
S. Em ±	0.16	0.63	0.76	0.93
CD@1%	0.67	2.61	3.14	3.84
MXS	NS	NS	NS	NS
SXL	NS	NS	NS	NS
MXL	NS	NS	NS	NS
MXSXL				
M1S1L1	16.59	65.73	79.06	96.82
M1S1L2	15.11	59.11	70.88	86.78
M1S2L1	16.48	65.43	78.85	96.75
M1S2L2	12.28	47.79	57.97	69.80
M2S1L1	18.20	71.20	85.37	104.53
M2S1L2	15.97	62.48	74.91	91.72
M2S2L1	17.65	69.05	82.79	101.37
M2S2L2	15.78	61.73	74.02	90.63
S. Em ±	0.32	1.26	1.52	1.86
CD @ 1%	1.33	5.22	6.27	7.67

Factor M : Potting mixtures Factor; S : Nutrient supplements
Factor L : Levels
M1 : Soil + FYMS1: OMF-NS1 CakeL1 : 100% equivalent RDP
M2 : Soil + OMF-PMS2: OMF-NS2 CakeL2 : 75% equivalent RDP

Note : OMF-PM- 10C:1RP, OMF-NS1 cake-1:2.75:1 and OMF-NS2 cake-1:2.5:1

Treatment receiving Soil + OMF-PM +100 per cent equivalent RDP through OMF-NS1 Cake (18.20, 71.20, 85.37 and 104.53, respectively) recorded significantly higher number of leaves per plant at 30, 60, 90 and 120 DAT and was on par with OMF-PM + 100 per cent equivalent RDP through OMF-NS2 Cake (17.65, 69.05, 82.79 and 101.37, respectively), followed by Soil + OMF-PM + 75 per cent equivalent RDP through OMF-NS1 Cake (15.97, 62.48, 74.91 and 91.72, respectively) and OMF-PM +75 per cent equivalent RDP through OMF-NS2 Cake (15.78, 61.73, 74.02 and 90.63, respectively). Significantly lower number of leaves per plant was noticed in Soil+FYM + 75 per cent equivalent RDP through OMF-NS2 Cake (12.28, 47.79, 57.97 and 69.80, respectively).

Internodal Length

The data pertaining to the effect of organo-mineral fertilizes on internodal length of tomato recorded at different growth stages (30, 60, 90 and 120 DAT) are presented in Table 4. There was a significant difference in internodal length due to application of organo-mineral fertilizers. At 30, 60, 90 and 120 DAT, significantly highest internodal length (6.64, 9.66, 10.47 and 12.37 cm, respectively) was recorded in Soil+OMF-PM than Soil+FYM. Whereas among nutrient supplements, OMF-NS1 Cake recorded significantly higher internodal length *i.e.,* 6.49, 9.45, 10.24 and 12.10 cm, respectively. Among levels, 100 per cent equivalent RDP level recorded significantly higher internodal length *viz.,* 6.82, 9.93, 10.76 and 12.70, respectively.

Internodal length increased significantly with the increase in days of crop growth. Highest internodal length was noticed in Soil + OMF-PM +100 per cent equivalent RDP through OMF-NS1 Cake (7.15, 10.41,11.27 and 13.32 cm, respectively) which was on par with Soil + OMF-PM +100 per cent equivalent RDP through OMF-NS2 Cake (6.93, 10.09, 10.93 and 12.92 cm, respectively) at 30, 60, 90 and 120 DAT. Treatment which received Soil + FYM +75 per cent equivalent RDP through OMF-NS2 Cake registered lowest internodal length (4.80, 7.15, 7.87 and 9.12 cm, respectively).

Table 4

Effect of organo-mineral fertilizers on internodal length at different growth stages of tomato

Treatment	Number of branches			
	30 DAT	60 DAT	90 DAT	120 DAT
Potting mixtures				
M1	5.98	8.75	9.51	11.18
M2	6.64	9.66	10.47	12.37
S. Em±	0.06	0.09	0.10	0.12
CD@1%	0.26	0.38	0.41	0.49
Nutrient supplements				
S1	6.49	9.45	10.24	12.10
S2	6.13	8.96	9.74	11.45
S. Em±	0.06	0.09	0.10	0.12
CD@1%	0.26	0.38	0.41	0.49
Levels				
L1	6.82	9.93	10.76	12.70
L2	5.80	8.48	9.22	10.85
S. Em±	0.06	0.09	0.10	0.12
CD@1%	0.26	0.38	0.41	0.49
MXS	NS	NS	NS	NS
SXL	NS	NS	NS	NS
MXL	NS	NS	NS	NS
MXSXL				
M1S1L1	6.61	9.62	10.43	12.32
M1S1L2	5.93	8.64	9.36	11.06
M1S2L1	6.59	9.59	10.40	12.23
M1S2L2	4.80	7.15	7.87	9.12
M2S1L1	7.15	10.41	11.27	13.32
M2S1L2	6.27	9.13	9.89	11.69
M2S2L1	6.93	10.09	10.93	12.92
M2S2L2	6.20	9.02	9.77	11.55
S.Em±	0.13	0.19	0.20	0.24
CD@1%	0.52	0.76	0.83	0.98

Factor M : Potting mixtures Factor; S : Nutrient supplements
Factor L : Levels
M1 : Soil + FYMS1: OMF-NS1 CakeL1 : 100% equivalent RDP
M2 : Soil + OMF-PMS2: OMF-NS2 CakeL2 : 75% equivalent RDP

Note : OMF-PM- 10C:1RP, OMF-NS1 cake-1:2.75:1 and OMF-NS2 cake-1:2.5:1

Growth parameters *viz.,* plant height, number of leaves per plant, number of branches per plant and internodal length were significantly higher in treatment that received OMF-PM + 100 per cent equivalent RDP through OMF-NS1 Cake. These results clearly indicated that the extent of increase in growth parameters that could be obtained with application of OMF-PM + 100 per cent equivalent RDP through OMF-NS1 Cake might be due to the higher amount of primary nutrients which were supplied from both OMF-PM and OMF-NS1 cake and initial requirement of N was met from the inorganic source and subsequent requirement of N through OMF-PM and OMF-NS1 cake which contained more amount of nitrogen and phosphorus and potassium compared to other assuring continuous N supply throughout growing period which favoured the consistent N uptake by plant at different growth stage favouring increase in growth parameters. The results are in accordance with the studies conducted on tomato crop by Ayeni (2010) and Kataoka *et al.* (2017) which provide strong evidence that organo mineral fertilization improves tomato crop performance through enhanced growth, yield, nutrient uptake and stress resilience.

The higher content of phosphorus in OMF-NS1 cake helped in stimulation of root growth which might have helped in better uptake of nutrients by the tomato crop which is depicted in increased number of branches which increased number of leaves per plant (Singh *et al.,* 2023). Potting mixture increased the microbial population by enhancing organic pool in soil on account of organo mineral fertilizers (Alabadan & Adeoye, 2019, Pooja & Satish, 2023 and Das *et al*., 2014).

Chlorophyll (SPAD values)

Chlorophyll content of tomato varied significantly due to application of organo-mineral fertilizers at 30, 60, 90 and 120 DAT of crop (Table 5). Soil + OMF -PM recorded significantly highest chlorophyll content (35.60, 41.55, 45.27 and 46.37, respectively). Whereas among nutrient supplements, OMF-NS1 Cake recorded significantly higher Chlorophyll *i.e.,*

Table 5

Effect of organo-mineral fertilizers on chlorophyll at different growth stages of tomato

Treatment	Number of branches			
	30 DAT	60 DAT	90 DAT	120 DAT
Potting mixtures				
M1	31.88	36.43	40.68	39.13
M2	35.60	41.55	45.27	46.37
S. Em±	0.34	0.39	0.43	0.44
CD@1%	1.39	1.62	1.77	1.80
Nutrient supplements				
S1	34.47	39.90	43.96	43.72
S2	33.01	38.08	41.99	41.78
S. Em±	0.34	0.39	0.43	0.44
CD@1%	1.39	1.62	1.77	1.80
Levels				
L1	35.32	40.92	44.91	45.94
L2	32.16	37.05	41.04	39.57
S. Em±	0.34	0.39	0.43	0.44
CD@1%	1.39	1.62	1.77	1.80
MXS	NS	NS	NS	NS
SXL	NS	NS	NS	NS
MXL	NS	NS	NS	NS
MXSXL				
M1S1L1	33.61	38.62	42.73	41.81
M1S1L2	32.93	37.64	42.36	40.06
M1S2L1	33.59	38.57	42.69	41.70
M1S2L2	27.40	30.88	34.95	32.96
M2S1L1	37.15	43.41	47.27	50.32
M2S1L2	34.20	39.93	43.49	42.69
M2S2L1	36.93	43.09	46.93	49.92
M2S2L2	34.11	39.76	43.37	42.55
S. Em±	0.67	0.78	0.86	0.87
CD@1%	2.78	3.23	3.54	3.60

Factor M : Potting mixtures Factor; S : Nutrient supplements
Factor L : Levels
M1 : Soil + FYMS1: OMF-NS1 CakeL1 : 100% equivalent RDP
M2 : Soil + OMF-PMS2: OMF-NS2 CakeL2 : 75% equivalent RDP

Note : OMF-PM- 10C:1RP, OMF-NS1 cake-1:2.75:1 and OMF-NS2 cake-1:2.5:1

34.47, 39.90, 43.96 and 43.72, respectively. Among levels, 100 per cent equivalent RDP level recorded significantly higher internodal length *viz.,* 35.32, 40.92, 44.91 and 45.94, respectively.

Treatment that received Soil + OMF-PM + 100 per cent equivalent RDP through OMF-NS1 Cake recorded greater chlorophyll content of 37.15 and 43.41, respectively at 30 and 60 DAT as compared to other treatments. At 90 and 120 DAT, the treatment that received Soil + OMF-PM + 100 per cent equivalent RDP through OMF-NS1 Cake recorded significantly highest chlorophyll content (47.27 and 50.32, respectively) followed by treatment with Soil + OMF-PM +100 per cent equivalent RDP through OMF-NS2 Cake (46.93 and 49.92, respectively) compared to other treatments. Application of Soil + FYM +75 per cent equivalent RDP through OMF-NS2 Cake recorded lower chlorophyll content at (27.40, 30.88, 34.95 and 32.96, respectively.

At all stages of plant growth, the interactions between potting mixtures and nutrient supplements, nutrient supplements and RDP levels, as well as potting mixtures and RDP levels, were found to be non-significant effect on all growth parameters.

Application of OMF-PM showed relatively higher chlorophyll content than only FYM applied treatment that recorded least chlorophyll content. The progressive improvement in tomato have resulted in better improvement, establishment, availability and uptake, primarily driven by enhanced soil organic matter and microbial activity. The incorporation of organic amendments significantly increases soil organic matter, nitrogen, phosphorus and potassium, thereby improving water and nutrient-holding capacity and creating a rich reservoir for plant uptake. This enriched soil environment supports vigorous vegetative growth, evidenced by increased plant height, number of leaves, number of branches and chlorophyll content factors tightly linked to enhanced SPAD values. Higher SPAD reading upon application of organo-mineral fertilizers might be due to increased additional supply of N, Mg and efficient absorption of N and Mg from soil which

are vital components of chlorophyll. Higher chlorophyll levels result in more efficient light absorption, larger photosynthetic capacity and elevated net photosynthetic rate, as well as improved stomatal conductance, intercellular CO and key chlorophyll fluorescence parameters. Higher SPAD reading upon application of organo-mineral fertilizers might be due to increased additional supply of N, Mg and efficient absorption of N and Mg from soil which are vital components of chlorophyll. The increase in chlorophyll content increased the photosynthetic activity of tomato which ultimately resulted in better growth crop. Similar increase in chlorophyll content and SPAD reading upon application of organo-mineral fertilizers was evidenced by Kumar *et al.* (2023) and Li *et al*. (2023).

Dry Matter Accumulation

The influence of organo-mineral fertilizers on shoot and fruit dry weight at harvest are presented in Fig. 2. Application of different potting mixtures, nutrient supplements and levels affected shoot and fruit dry weight significantly. Application of Soil+ OMF-PM recorded significantly higher shoot and fruit dry weight of 72.48 g plant^{-1} and 7.70 g fruit^{-1}, respectively, than Soil+ FYM (64.54 g plant^{-1} and 6.76 g fruit^{-1}, respectively). Application OMF-NS1 Cake recorded higher shoot and fruit dry weight of 70.95 g plant^{-1} and 7.54 g fruit^{-1}, respectively, than OMF-NS2 Cake (66.07 g plant^{-1} and 6.91 g fruit^{-1}, respectively). 100 per cent equivalent RDP recorded significantly higher values 72.04 and 7.81g plant^{-1}, respectively as compared to 75 per cent RDP level (64.98 g plant^{-1} and 6.65 g fruit^{-1}, respectively).

The interactions between potting mixtures and nutrient supplements, nutrient supplements and RDP levels and potting mixtures and RDP levels showed no significant effect on shoot dry weight. However, for fruit dry weight, the interactions between potting mixtures and nutrient supplements, as well as nutrient supplements and RDP levels, were significant, while the interaction between potting mixtures and RDP levels remained non-significant. Among the different treatment

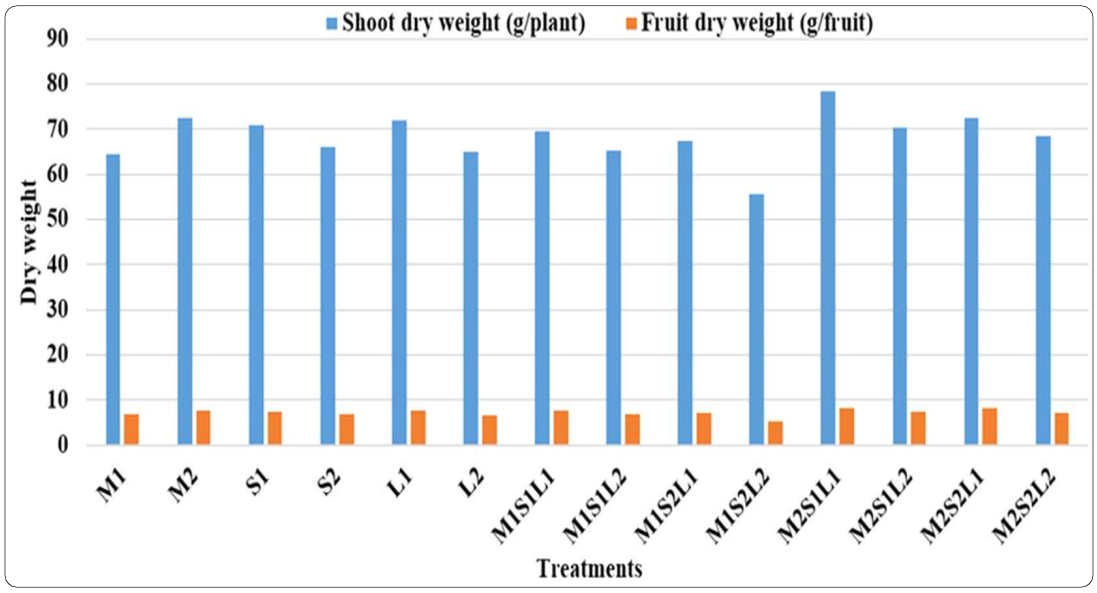

Fig. 2 : Effect of organo-mineral fertilizers on dry weight of shoot and fruit of tomato

Treatment details:

Factor M : Potting mixtures	Factor S : Nutrient supplements	Factor L : Levels
M1 : Soil + FYM	S1 : OMF - NS1 Cake	L1 : 100% equivalent RDP
M2 : Soil + OMF-PM	S2 : OMF - NS2 Cake	L2 : 75% equivalent RDP

combinations, application of OMF-PM +100 per cent equivalent RDP through OMF-NS1 Cake recorded maximum shoot and fruit dry weight (78.45 g plant^{-1}and 8.24 g fruit^{1}, respectively) and found to be on par with treatment (72.56 g plant^{-1} and 8.11 g fruit^{-1}, respectively), which received OMF-PM +100 per cent equivalent RDP through OMF-NS2 Cake and lowest (55.68 g plant^{-1} and 5.20 g fruit^{-1}, respectively) was recorded in Soil + FYM +75 per cent equivalent RDP through OMF-NS2 Cake. Interaction of these two factors on plant was found to be non-significant at all stages of crop.

The synergistic application of organo mineral fertilizer (OMF PM) alongside a 100 per cent rate of RDP through OMF NS1 Cake significantly boosted dry matter accumulation in tomato. This outcome stems from the consistent and sustained delivery of both organic and mineral nutrients at crucial vegetative and reproductive stages. The organic matrix within OMF PM provides immobilized nutrients that are gradually mineralized by soil microorganisms, ensuring a prolonged nutrient release. Simultaneously, the mineral component guarantees the immediate availability of essential elements like phosphorus. Together, these components create a rich, balanced nutrient reservoir that supports continuous foliage expansion and robust root development. As roots explore the soil, they absorb nutrients more efficiently, stimulating the construction of a vigorous photosynthetic apparatus. This promotes rapid leaf area development and strong canopy establishment, which enhances light interception and overall plant vigor. Higher nutrient availability during flowering and further intensifies biomass production. Additionally, the microbial activity associated with OMF PM improves nutrient cycling and soil structure, creating an optimal environment for sustained plant growth. The result is a steady, elevated rate of dry matter accumulation, with more biomass being partitioned into economically valuable plant parts. Moreover, this combined fertilization strategy can enhance water-use efficiency, as healthier canopies reduce soil evaporation. It also boosts the plant's resilience to stress by supplying essential nutrients during critical growth phases. Overall, employing OMF PM with full-dose RDP through OMF NS1 Cake establishes a dynamic, nutrient-rich

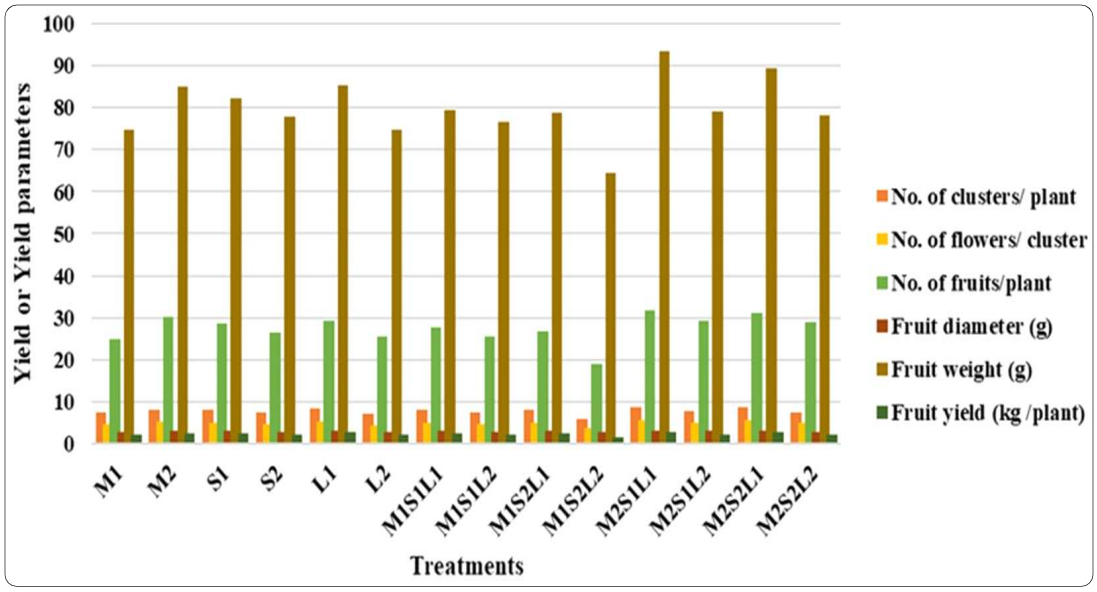

Fig. 3: Effect of organo-mineral fertilizers on yield and yield prameters of tomato

Treatment details:

Factor M: Potting mixtures	Factor S: Nutrient supplements	Factor L: Levels
M1: Soil+FYM	S1: OMF-NS1 Cake	L1: 100% equivalent RDP
M2: Soil+ OMF-PM	S2: OMF-NS2 Cake	L2: 75% equivalent RDP

system that drives continuous growth and higher yields. Similar results were also obtained by Yadav *et al.* (2024) and Singh *et al.* (2023) showcase that combining vermicompost with reduced NPK enhanced shoot and fruit dry weight over controls.

Effect of Organo-Mineral Fertilizers on Yield Parameters and Yield of Tomato at Harvest

Yield Parameters

The data on yield attributes of tomato *viz.*, number of clusters per plant, number of flowers per cluster and number of fruits per plant is depicted in Fig. 3.

Organo-mineral fertilizers application had a significant effect on the number of clusters per plant, number of flowers per cluster and the number of fruits per plant. The Soil + OMF-PM treatment resulted in the highest values for these parameters, with 8.24, 5.27 and 30.24, respectively which were significantly higher than those observed in the Soil + FYM (7.41, 4.56 and 24.84, respectively) treatment. Among the different nutrient supplements, OMF-NS1 Cake showed a notable increase, recording 8.06, 5.04 and 28.57, respectively over OMF-NS2 Cake (7.59, 4.79 and 26.51, respectively). Additionally, the application of the 100 per cent equivalent RDP level led to the most pronounced improvement, with 8.41, 5.32 and 29.37, respectively compared to 75 per cent RDP level (7.24, 4.51 and 25.71, respectively). Interaction of these two factors on plant was found to be significant in case of number of fruits per plant whereas rest were non-significant.

The application of organo-mineral fertilizers improved yield attributes, as demonstrated by Soil + OMF-PM + 100 per cent equivalent RDP through OMF-NS1 Cake, which recorded 8.78 clusters per plant, 5.67 flowers per cluster and 31.67 fruits per plant. These results were statistically comparable to those of Soil + OMF-PM + 100 per cent equivalent RDP through OMF-NS2 Cake, which achieved 8.65, 5.61 and 31.67, respectively. In contrast, the lowest values were observed in Soil + FYM + 75 per cent equivalent RDP through OMF-NS2 Cake, with 5.95, 3.69 and 19.15, respectively.

Fruit Diameter

Fruit diameter did not vary significantly with the application of organo-mineral fertilizers. However, among the potting mixtures, Soil + OMF-PM treatment recorded the highest values, with a fruit diameter of 3.0 cm. Among the treatments, higher fruit diameter (3.25 cm) was recorded in Soil + OMF-PM + 100 per cent equivalent RDP through OMF-NS1 Cake which was followed by OMF-PM + 100 per cent equivalent RDP through OMF-NS2 Cake, (3.13 cm). The lower fruit diameter and fruit weight (2.85 cm) was recorded in Soil + FYM + 75 per cent equivalent RDP through OMF-NS2 Cake.

Fruit Weight

The Soil + OMF-PM treatment recorded the highest values, with a fruit weight of 85.09 g, significantly surpassing those in the Soil + FYM treatment (74.79 g). Among nutrient supplements, OMF-NS1 Cake exhibited a noticeable increase, achieving a fruit weight of 82.18 g than OMF-NS2 cake (82.18 g). Furthermore, the 100 per cent equivalent RDP level application resulted in the most substantial improvement, with values reaching 85.28 g when compared to 75 per cent equivalent RDP level (74.60 g). Interaction of these two factors on fruit parameters was found to be significant in case of fruit weight.

Fruit weight varied significantly with the application of organo-mineral fertilizers. However, higher fruit weight (93.45 g) was recorded in Soil + OMF-PM + 100 per cent equivalent RDP through OMF-NS1 Cake which was followed by OMF-PM + 100 per cent equivalent RDP through OMF-NS2 Cake, (89.45 g). The lower fruit weight (64.45 g) was recorded in Soil + FYM + 75 per cent equivalent RDP through OMF-NS2 Cake.

The increase in yield parameters *viz*., number of clusters per plant, number of flowers per cluster and number of fruits per plant, fruit diameter and fruit weight in the treatment applied with OMF-PM + 100 per cent equivalent RDP through OMF-NS1 Cake, might be due to good soil physical properties such as improved structure, porosity, reduced bulk density

and greater organic matter content which directly boosted the soil's water retention and nutrient-holding capacities. Improved aggregate stability facilitated better root penetration and more extensive root systems, enabling plants to explore moisture and nutrients more effectively. With a steadier supply of essential nutrients, plants can support a larger number of reproductive structures, leading to more clusters and more flowers per cluster. This nutrient-rich environment also curbs nutrient stress during pollination, significantly reducing flower drop and translating into a higher fruit set per plant. As more fruits develop, they benefit from the sustained nutrient and moisture supply, growing larger in diameter and heavier in weight. The increase in fruit size itself creates favourable micro environments for further resource allocation, reinforcing the positive reaction on fruit development.

The presence of organic matter from both OMF-PM and OMF-NS1 Cake also fosters microbial activity that enhances nutrient cycling and stabilizes soil moisture. These microbes mineralize organic nutrients gradually, keeping the soil environment dynamic and fertile throughout the fruiting period. Combined with optimal soil moisture, this microbial influence supports prolonged and steady nutrient availability directly to developing fruits. As a result, each fruit not only attains a greater physical dimension but also a denser internal structure, yielding heavier fruit weight. This leads to even better fruit size, weight and fruit number in subsequent seasons illustrating the long-term fertility gains achieved through this combined fertilization approach. The results are in accordance with the studies by Puri *et al.* (2021), Rady (2012) and Sitzmann *et al.* (2024) highlighted how innovative organo-mineral fertilizer strategies enhance yield outcomes across conditions.

Fruit Yield

Fruit yield of tomato under OMF application is presented in Fig. 3. The treatment with Soil + OMF-PM resulted in the highest fruit yield of 2.62 kg plant^{-1} when compared to Soil + FYM (2.21 kg plant^{-1}). Among the different nutrient supplements, OMF-NS1 Cake registered the highest fruit yield of 2.49 kg plant^{-1} as COMPARED TO OMF-NS2 Cake (2.33 kg plant^{-1}). Additionally, the application of nutrients at a 100 per cent equivalent RDP level led to the highest fruit yield of 2.68 kg plant^{-1} compared to 75 per cent equivalent RDP (2.15 kg plant^{-1}). The interaction between these two factors had a significant impact on fruit yield.

The treatment involving Soil + OMF-PM + 100 per cent equivalent RDP through OMF-NS1 Cake resulted in a significantly higher fruit yield (2.97 kg plant^{-1}) and was found to be statistically on par OMF-PM + 100 per cent equivalent RDP through OMF-NS2 Cake (2.79 kg plant^{-1}). Additionally, it was significantly higher than the yield observed in Soil + OMF-PM + 75 per cent equivalent RDP through OMF-NS1 Cake (2.36 kg plant^{-1}) and OMF-PM + 75 per cent equivalent RDP through OMF-NS2 Cake (2.34 kg plant^{-1}). The lowest fruit yield was recorded in the treatment with Soil + FYM + 75 per cent equivalent RDP through OMF-NS2 Cake (1.69 kg plant^{-1}).

Application of Soil + OMF-PM treatment recorded the highest fruit yield (2.62 kg plant^{-1}), significantly outperforming the traditional Soil + FYM treatment. This superior performance can be attributed to the balanced nutrient composition and improved soil physical and biological properties associated with OMF-PM. The enhanced nutrient-use efficiency and continuous nutrient release from the OMF-PM blend likely supported better root development, vegetative growth and ultimately higher fruit set and yield. Among the nutrient supplements, OMF-NS1 Cake registered the highest yield (2.49 kg plant^{-1}). The improved performance of NS1 may be due to its rich nutrient profile, better solubility and synchronized nutrient release, which collectively support sustained plant growth and reproductive development. The supplement's favourable impact on biochemical parameters like sugars, proteins and antioxidants might have contributed indirectly to improved yield the 100 per cent equivalent RDP application significantly increased fruit yield (2.68 kg plant^{-1}) compared to the 75 per cent RDP level. This highlights

the critical role of adequate nutrient supply in achieving optimum physiological functioning and reproductive output. A reduced nutrient level (75 per cent RDP) may have imposed mild nutrient stress, limiting fruit development and lowering overall yield potential.

Additionally, the rich organic substrate fosters a thriving microbial community-boosting populations of bacteria, fungi and actinomycetes that enhance nutrient mobilization, soil enzyme activity and structural stability. These beneficial microbes break down complex organic compounds into bioavailable forms and produce phytohormones that stimulate root and shoot growth, further promoting metabolic activity within the plant. Enhanced root proliferation improves nutrient and water uptake, even under stress conditions like drought, leveraging both physical soil improvements and symbiotic microbial functions.

These combined soil and plant factors synergistically elevate the photosynthetic capacity, resulting in a higher supply of carbohydrates. With increased carbohydrate production, the plants can sustain a larger number of flowering clusters and flowers per cluster, while nutrient stability during flowering reduces flower drop. This leads to a greater fruit set per plant. As fruits develop, they benefit from the steady translocation of carbohydrates and continuous nutrient supply, which supports increased fruit diameter and weight. Large fruits have occurred due to increased photosynthetic area and translocation of photosynthates in plants which subsequently accelerated the formation of more number of large sized fruits with increased fruit diameter resulting in increase in fruit weight as quoted by Akintokun *et al.* (2020), Nath *et al.* (2023) and Ayeni *et al.* (2017). The cumulative impact is reflected in substantial yield gains.

The findings of the present study clearly demonstrate the positive impact of organo-mineral fertilizers on the growth and yield of tomato (*Lycopersicum esculentum* L.). Significant improvements in plant height, biomass accumulation and fruit yield were observed, indicating enhanced soil fertility and nutrient availability. The integrated application of organic and mineral components contributed to better nutrient synchrony with crop demand, ultimately leading to improved plant performance. These findings highlight the potential of organo-mineral fertilizers as an effective and sustainable strategy for optimizing tomato production under urban horticulture because this one product can supply all the essential nutrients required for the plant growth and development.

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Effect of Priming Treatment on Physical and Physiological Properties in Aged Seeds of Contrast Colored Soybean (*Glycine max* (L.) Merrill) Genotypes

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Abstract

Priming is a pre-sowing, controlled hydration treatment, permits pre-germinative metabolic activity, but prevent radical emergence. The study revealed that among elite genotypes of soybean, JS 335 (white seed coat) and EC 993950 (black seed coat) performed significantly better in terms of germination percentage, root length, shoot length, mean seedling length, mean seedling dry weight, seedling vigor index-I and II. Membrane deterioration assessed through electrical conductivity (EC) of seed leachates showed initial EC values, 15.11 and 11.57 µS cm^{-1} g^{-1} for JS 335 and EC 993950, respectively. After 72 hours of ageing, EC values increased to 52.32 and 42.62 µS cm^{-1} g^{-1} indicating increased electrolyte leakage. Upon seed priming, notably with osmo priming using PEG at 1.1 MPa for 6 hours, the EC values were 33.03 and 28.62 µS cm^{-1} g^{-1}) and for 12 hours of priming, 34.80 and 30.69 µS cm^{-1} g^{-1} in JS 335 and EC 993950, respectively. Fresh seeds showed higher dehydrogenase (TDH) activity (1.461 and 1.828 OD @ A480 nm) than aged seeds (0.794 and 1.150 OD @ A480 nm). Priming improved TDH activity, with the highest values observed under PEG priming for 6 hours (1.181 and 1.541 OD), followed by 12 hours (1.100 and 1.503 OD). White seed coat genotypes were more susceptible to ageing than black ones. The observed differences in seed quality, EC and TDH indicate better membrane integrity in black seed coat genotypes, likely due to the presence of proanthocyanidins with antioxidant activity, which helps to reduce membrane damage.

Keywords : Priming, Germination, Electrical conductivity, Dehydrogenase, Ageing

Soybean [*Glycine max* (L.) Merrill] is a crucial global agricultural commodity with significant economic importance and increasing production. It has an exceptional protein content of about 40 per cent and the third-highest oil content at around 20 per cent. Leading soybean producers are Brazil, the United States, Argentina, China and India. This versatile legume is used in animal feed, human nutrition, cosmetics, fibers and biodiesel production. Soybean was selected owing to their diverse characteristics related to storage. Soybean, both a pulse and oilseed is very sensitive to storage. Also, a dicotyledon will provide an opportunity to study the difference in seed coat properties and storage behavior during ageing and priming in diverse genotypes. Seed ageing is an irreversible and inexorable process of a progressive decrease in vigor ultimately leading to the loss of seed viability (Arc *et al.,* 2011 and Lehner *et al.,* 2008). The rate

of seed ageing depends upon the genotype/ species, the conditions prevailing during storage like moisture content, temperature, humidity and seed composition (Roberts, 1973). It has been reported that high moisture content and high temperature accelerate seed deterioration (Ellis & Hong, 1991 and Goel *et al.,* 2003) based on which seeds are accelerated to artificially age by exposing them to high humidity and temperature of about 40-45 °C and 100 per cent RH (Delouche & Baskin, 1973). The moisture absorption during ageing would lead to swelling of seeds altering the seed size parameters. So, the morphometric changes like seed perimeter, area, diameter, roundness, roughness, elongation and compactness after ageing would indicate the rate of seed deterioration (Nethra *et al*., 2005). It is reported that higher moisture content in seeds after aging leads to faster deterioration resulting in lesser germination. It also correlates to increase in size and decrease in vigor (Srii *et al*., 2021). Accelerated ageing is shown to induce many deteriorative changes to seeds during storage like genetic damage, protein degradation, enzyme inactivation and loss of membrane integrity (Arc *et al*., 2011; Bailly, 2004; Bailly *et al.*, 1996, 2002; Merritt *et al.,* 2003; Ratajczak & Pukacka, 2005; Ratajczak *et al.,* 2015 and Wang *et al*., 2011) which ultimately lead to reduced germination (Walters, 1998) and loss of viability. Previous studies (Renuka *et al.*, 2022) have reported that ageing is irreversible degenerative change which affect seed germination and vigor.

Seed germination of desiccated quiescent seed usually occurs as a three-step process (Bewley, 1997 and Weitbrecht *et al.,* 2011), commencing with imbibition and terminating with radical protrusion. However, the rate of water absorption by seeds during this phase of imbibition depends on the permeability properties of the seed coat like its hydrophobicity, thickness, porosity and integrity. It is reported that seed coats with cracks or pores imbibe water rapidly compared to intact seed coats (Duke & Kakefuda, 1981; Duke *et al*., 1983; Duke *et al*., 1986; Oliveria *et al*., 1984; Powell & Matthews, 1979 and Tully *et al*., 1981). However, in *Fabaceae*, hard seeds with impermeable seed coats pose a problem for phase I of imbibition, a critical period for germination as 80 per cent of water absorption is reported to occur during this phase (Souza and Marcos-Filho, 2001). It is known that the seeds lose membrane integrity upon ageing or by other mechanical handling procedures during storage altering the seed coat's permeability properties. Veselova and Veselovsky (2003) reported that aged pea seeds showed imbibitional injury leading to the production of abnormal seedlings, which they proposed to be due to permeability changes in the seed coat to water. However, the changes brought about by accelerated ageing on seed coat structure result in altered permeability properties.

Maintenance of seed quality from harvest to planting is of utmost important in the seed industry. The seed quality mainly depends on various factors *viz.,* genotypes, provenance, production practices and prevailing environmental conditions during seed production, post-harvest operation, post-harvest handling, storage environment, initial quality of the seeds etc. The alternative to overcome these problems is through seed invigoration technology. Invigoration is a way to improve the physiological quality of seeds, especially seed vigor, through chemical treatment (Parera and Cantliffe, 1994). In the recent past, many studies proved the efficiency of seed priming technique to enhance the germination rate, uniformity of seedling growth and to reduce the time of seedling emergence (Harris *et al*., 2005). Seed priming is a pre-sowing treatment in which seeds are soaked in water and or other chemicals for a definite period of time and then dried back to original moisture content before sowing (Taylor *et al*., 2017). Seed priming stimulates various biochemical changes in seed which are essential to commence germination process besides improving its physiology (Ajouri *et al*., 2004). It synchronizes germination after breaking dormancy, diminishes the lag time required for imbibition, hydrolyses or metabolizes inhibitors, activates enzymes, mobilizes reserved food and enhances embryonic tissue outgrowth. Priming is able to repair the age related cellular and sub-cellular damage of low vigor seeds that may accumulate during seed development (Mc-Donald, 2000). In priming, seeds are soaked in different solutions with high osmotic

potential, prevents the seeds from absorbing enough water for radicle protrusion, thus suspending the seeds in the lag phase (Taylor *et al*., 1998).

Another major reason to opt for this crop is the fact that there are different color genotypes available in this crop which help us in exploring the difference between colored genotypes. Previous studies have reported that the cultivars with dark seed coat in chickpea (Gvozdeva and Zhukove, 1971), soybean, snap bean (*Phaseolus vulgaris*) (Prasad and Weigle, 1976), french bean (Powell *et al.*, 1986) were less permeable and exhibit longer life span than the cultivars with light seed coat color. Black-seeded soybeans exhibit superior physiological quality and storage longevity compared to white-seeded ones. This investigation was conducted to study the best priming treatment for seed quality parameters. The performance is investigated in this experiment by the use of seeds taken from aged seed lot and priming treatments to aged seeds to increase the germination percentage. Keeping these things in view, the present investigation entitled 'Effect of priming treatment on physical and physiological properties in aged seeds of contrast colored soybean (*Glycine max* (L.) Merrill) genotypes' was carried out.

Material and Methods

Soybean seeds received from All India Coordinated Research Project on Soybean, Zonal Agricultural Research Station, University of Agricultural Sciences, Bangalore were multiplied in the fields of Seed Technology Research Center, AICRP on Seeds (Crops), Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bangalore, which is situated at an altitude of 13^0.15'.N and longitude of 77^0.32'.E. during 2019-2022. The lab experiments were conducted at Seed Technology Research Center, AICRP on Seeds (Crops), Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bangalore. The detailed materials and methods adopted for the conduct of different experiments are described below.

Seed Material

Initially 40 soybean genotypes were evaluated for germination. Based on germination percentage three black and three white seed coat colored genotypes were selected for further studies. The genotypes used for the study were, the black seed coat genotypes G1: EC993608, G2: EC993850, G3: EC993950 and white seed coat genotypes G4: EC993703, G5: EC993937, JS 335.

Accelerated Ageing

Artificial ageing was performed according to ISTA guidelines (Anonymous, 2013). The moisture content of the fresh seed samples was determined by the hot air oven method (Anonymous, 2013) before proceeding with accelerated ageing. For artificial ageing (AA), the plastic boxes were first sterilized with 5 per cent sodium hypochlorite, dried and then each was filled with 40±1.0 ml of distilled water. 42 grams of seeds were placed on the screen in one layer deep to ensure an even uptake of moisture from the humid environment and the lid was placed on each box. The boxes were placed on the shelves of the ageing chamber (Manufacturer: Thermo Scientific, Model: IGS 60/100/180) allowing air space of 2.5 cm between boxes to assure temperature uniformity. The temperature was set to 41±0.3p °C and 100 per cent RH and was monitored at regular intervals. After 48 and 72 h of ageing, the AA boxes were removed from the chamber. The seed moisture content of seeds after ageing was measured using a digital moisture meter.

Priming Treatments

Four priming treatments were included for studies. T1: Fresh seeds, T2: Aged seeds, T3: Hydro priming for 8 hours at 25p °C, T4: Ascorbic acid priming 8 hours at 25p °C, T5: Osmo priming PEG at 1.2MPa for 12 hours at 25p °C, T6: Osmo priming PEG at 1.2MPa for 6 hours at 25p °C.

Germination (%)

Seed germination test was performed within one hour after removal from the ageing chamber. 100 seeds in four replications for each ageing treatment and control (fresh, non-aged seeds) were taken for germination test by paper method at 25p °C. The first and final count was taken on the 5th and 8th day and seedlings were evaluated as per International Seed Testing

Association guidelines (2013) considering the per centage of normal seedlings to be germinated.

Root Length (cm)

From the standard germination test, ten normal seedlings from each treatment in four replications were randomly selected on the day of final count. The length of root was measured from collar region to tip of the primary root of the seedling and expressed in centimeters.

Shoot length (cm)

From the standard germination test, ten normal seedlings from each treatment in four replications were randomly selected on the day of final count. The length of shoot was measured from collar region to the tip of shoot of the seedling and expressed in centimeters.

Mean Seedling Length (cm)

On 8th day of germination test, ten normal seedlings were taken randomly from four replications of each treatment and measured from the tip of primary root to the tip of apical shoot. The average of ten seedlings is expressed as mean seedling length in centimeters.

Mean Seedling Dry Weight (mg)

Ten normal seedlings used for measuring shoot and root length were taken in a butter paper and dried in hot air oven, at 80 °C temperature for 17 hours. Then the seedlings were removed and allowed to cool for 45 minutes before weighing on an electrical balance. The average seedling dry weight is expressed in milligram (mg).

Seedling Vigor Indices

To calculate the seedling vigor index-I (SVI-I), the seedling length (root and shoot) of five randomly selected seedlings in each replicate was measured while taking the final count of seeds in the germination test. The vigor index was calculated by multiplying germination (%) and seedling length (cm).

Seedling vigor index I = Germination per cent x Mean seedling length

To calculate the seedling vigor index-II (SVI-II), the seedling dry weight of five randomly selected seedlings in each replicate was measured while taking the final count of seeds in the germination test. The vigor index was calculated by multiplying germination (%) and seedlingdry weight.

Seedling vigor index II = Germination per cent x Seedling dry weight

Tetrazolium Viability Testing

The viability testing of seeds was performed for 50 seeds in two replicates for each ageing treatment using Tetrazolium (Tz) staining. For soybean, the seeds were soaked in distilled water for 18 h followed by the removal of the seed coat and then the seeds were soaked without seed coat in 1 per cent Tz solution for 3 h at 30p °C in dark. The uptake of stain was evaluated as per International Seed Testing Association guidelines.

Electrical Conductivity

Membrane deterioration was measured by an electrical conductivity (EC) test as per the modified ISTA method (Anonymous, 2013). Fifty seeds in two replicates in each ageing treatment along with control were soaked in 250 ml of distilled water for 24 h at 20p °C and the conductivity of the elute was measured using a conductivity meter (Manufacturer: Systronics, Model: 306).

Total Dehydrogenase

The total dehydrogenase activity was measured by the method suggested by Kittock and Law (1968). The seeds used in Tz viability testing were washed thoroughly with distilled water and the red-colored formazan from the stained regions was extracted by soaking in 5 ml of 2-methoxy ethanol for 8 h in an airtight container. The extract was decanted and the color intensity was measured in Spectrophotometer (Model Mini Spec 17) at 480 nm with a suitable blank (Methoxy ethanol). The total dehydrogenase activity (TDH) was expressed as absorbance. The significance of values for different sample groups of two genotypes

was analyzed using ANOVA (with single factor) with post hoc Tukey Krammer test using SPSS software.

Results and Discussion

Results showed no difference in seed viability after ageing in all genotypes with all seeds being viable (100 % viability), while the germination per cent varied between genotypes and also within the aged seeds and primed seeds. The initial germination per cent of 95.67 and 90.33 observed in fresh seeds was reduced to 64.67 and 75 per cent after 72 h of ageing in white (JS 335) and black (EC 993950) genotypes, respectively. When the seeds were subjected to different priming treatments the germination per cent increased to 70 and 80 in osmo priming-PEG at 1.1MPa for 6 hrs, followed by osmo priming-PEG at 1.1MPa for 12 hrs (69% and 78.33%) in white seed coat (JS 335) and black seed coat (EC 993950) genotypes, respectively (Table 1). Significant differences were observed in seed germination per cent after ageing and also between genotypes at $p<0.01$. Though white seed coat genotypes had 100 per cent viability, upon aging for 72 h germination per cent was reduced to 63.33 per cent from an initial 95.67 per cent in control (fresh) seeds. But priming of the aged seeds increased the germination (70%). The black seed coat genotypes also had 100 per cent viability, but the germination per cent was reduced to 73 per cent in seeds aged for 72 h from initial 90.33 per cent. The priming of seeds increased the germination (80%). The differences in germination per cent reduction after ageing between the genotypes is due to the increased fraction of abnormal seedlings produced in white genotypes, while black genotypes, due to hard seed coat, are resistant to damage caused upon ageing (Satyasrii *et al*., 2022). Seeds had 100 per cent viability even after ageing but the deteriorative changes that occurred due to ageing were reflected as abnormal seedlings. It was reported in peas and soybean that accelerated ageing increased the proportion of abnormal seedlings which reduced the germination per cent of aged seeds (Veselova and Veselovsky, 2003 and Rastegar *et al*., 2011). These research findings are in line with findings of Ghobadi *et al.* (2012), who recorded higher germination in primed seeds. The seedlings produced were healthy and vigorous due to gibberellin production and reserve mobilization (Zhu *et al*., 2010).

Mean Seedling Length

Results showed that, mean seedling length (34.97 cm and 37.90 cm) in fresh seeds, after 72 h of ageing it

Table 1

Influence of ageing and priming treatments on seed germination (%)

	EC993608 (G1)	EC993850 (G2)	EC993950 (G3)	EC993703 (G4)	EC993937 (G5)	JS 335 (G6)	Mean
Fresh/control (T1)	88.67	89.67	90.33	94.33	94.67	95.67	92.22
Ageing for 72 hrs (T2)	73.00	73.33	75.00	63.33	63.67	64.67	68.83
Hydro priming (T3)	75.33	76.67	77.67	65.67	66.00	67.00	71.39
Vit. C priming (T4)	76.00	77.33	78.00	66.33	67.00	68.33	72.17
PEG for12 hrs (T5)	77.00	77.67	78.33	66.67	67.33	69.00	72.67
PEG for 6 hrs (T6)	78.33	79.00	80.00	67.67	68.67	70.00	73.94
MEAN	78.06	78.94	79.89	70.67	71.22	72.44	
	SEM	SED	CD				
TRT	0.349	0.494	1.306				
CONC	0.349	0.494	1.306				
T*G	0.855	1.210	3.200				
CV	1.97						

TABLE 2

Influence of ageing and priming treatments on mean seedling length (cm)

	EC993608 (G1)	EC993850 (G2)	EC993950 (G3)	EC993703 (G4)	EC993937 (G5)	JS 335 (G6)	Mean
Fresh/control (T1)	34.65	34.70	37.90	33.80	34.30	34.97	35.05
Ageing for 72 hrs (T2)	28.66	29.33	30.95	26.16	26.95	27.80	28.31
Hydro priming (T3)	29.35	30.74	32.09	28.27	29.22	30.18	29.98
Vit. C priming (T4)	31.59	32.99	33.87	30.09	30.89	31.99	31.90
PEG for12 hrs (T5)	33.63	35.00	35.70	31.87	32.68	33.50	33.73
PEG for 6 hrs (T6)	35.53	36.98	37.59	33.79	34.90	35.59	35.73
MEAN	32.23	33.29	34.68	30.66	31.49	32.34	
	SEM	SED	CD				
TRT	0.051	0.072	0.191				
CONC	0.051	0.072	0.191				
T*G	0.125	0.177	0.468				
CV	0.67						

was decreased (27.80 cm and 30.95 cm) in white (JS 335) and black (EC 993950) genotypes, respectively. When the seeds are subjected to different priming treatments it increased to 35.59 cm and 37.59 cm in osmo priming-PEG at 1.1MPa for 6 hrs, followed by osmo priming-PEG at 1.1MPa for 12 hrs (33.50 cm and 35.70 cm) in white seed coat (JS 335) and black seed coat (EC 993950) genotypes, respectively (Table 2). Results showed that, the higher shoot length was observed (16.30 cm and 17.35 cm) in fresh seeds, after 72 h of ageing it was decreased (13.10 cm and 14.49 cm) in white seed coat (JS 335) and black seed coat (EC 993950) genotype, respectively. When the aged seeds were subjected to different priming treatments, it was increased to 16.79 cm and 17.75 cm in osmo priming-PEG at 1.1MPa for 6 hrs, followed by osmo priming-PEG at 1.1MPa for 12 hrs (15.93 cm and 16.93 cm) in white seed coat (JS 335) and black seed coat (EC 993950) genotype, respectively. The higher root length was observed

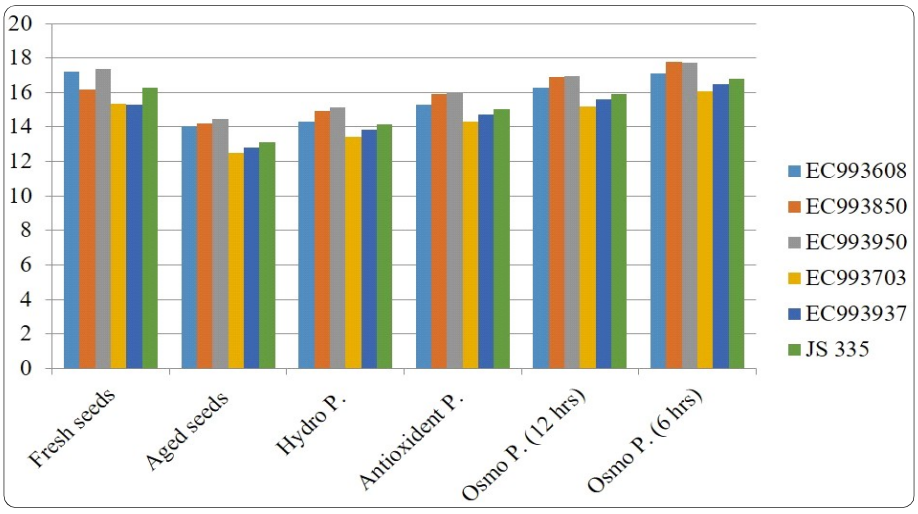

Fig. 1 : Influence of aging and priming treatments on shoot length (cm)

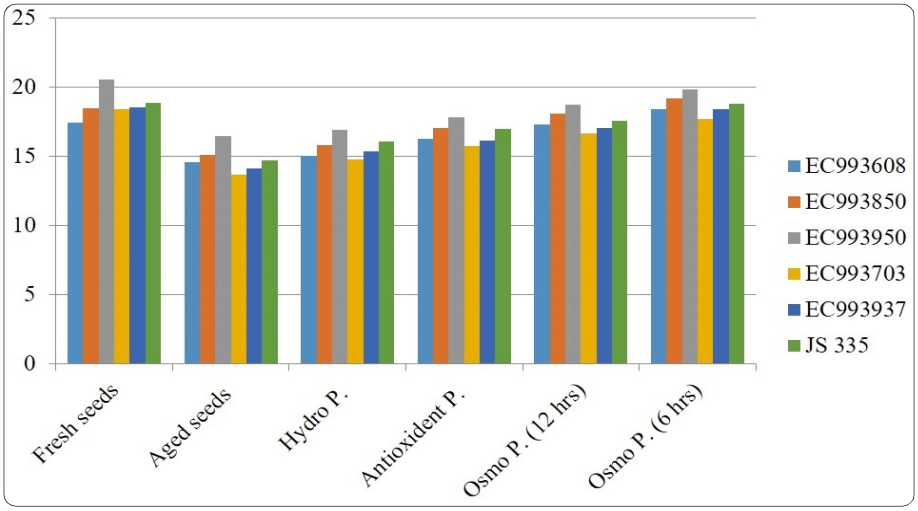

Fig. 2 : Influence of aging and priming treatments on root length (cm)

(18.97 cm and 20.55 cm) in fresh seeds, after 72 h of ageing it was decreased (14.70 cm and 16.46 cm). When the seeds are subjected to different priming treatments it was increased to 18.80 cm and 19.84 cm in osmopriming-PEG at 1.1MPa for 6 hrs, followed by osmopriming-PEG at 1.1MPa for 12 hrs (17.57 cm and 18.77 cm) in white seed coat (JS 335) and black seed coat (EC 993950) genotype respectively (Fig. 1 and 2). Our results are in conformity with the account of Guan *et al.* (2009) who found that priming increases the seed and seedling growth parameters. Basra *et al.* (1989) revealed that vit - C induced the seed germination percentage, radical length of seeds and seedling vigor index of soybean, pea and tomato seeds.

Mean Seedling Dry Weight

The initial mean seedling dry weight of 51.13 mg and 58.32 mg in fresh seeds, decreased to 35.34 mg and 43.37 mg after 72 h of ageing in white (JS 335) and black (EC 993950) genotypes, respectively. When the seeds were subjected to different priming treatments,

TABLE 3

Influence of ageing and priming treatments on mean seedling dry weight (mg)

	EC993608 (G1)	EC993850 (G2)	EC993950 (G3)	EC993703 (G4)	EC993937 (G5)	JS 335 (G6)	Mean
Fresh/control (T1)	56.26	57.16	58.32	48.23	49.64	51.13	53.46
Ageing for 72 hrs (T2)	40.25	40.80	43.37	30.87	32.99	35.34	37.27
Hydro priming (T3)	45.25	46.52	47.82	37.12	37.99	39.65	42.39
Vit. C priming (T4)	46.97	48.40	51.42	38.98	40.04	43.06	44.81
PEG for12 hrs (T5)	47.51	49.13	52.73	39.15	41.16	44.36	45.67
PEG for 6 hrs (T6)	48.87	49.43	54.44	40.50	42.06	46.07	46.89
MEAN	47.52	48.57	51.35	39.14	40.64	43.27	
	SEM	SED	CD				
TRT	0.105	0.149	0.394				
CONC	0.105	0.149	0.394				
T*G	0.258	0.365	0.965				
CV	0.99						

the mean seedling dry weight increased to 46.07 mg and 54.44 mg in osmo priming-PEG at 1.1MPa for 6 hrs, followed by osmo priming-PEG at 1.1MPa for 12 hrs (44.36 mg and 52.73 mg) in white seed coat (JS 335) and black seed coat (EC 993950) genotypes, respectively (Table 3). Priming promotes carbohydrates present in the seeds to be readily utilized for the cell division. Consequently it leads to increase in seedling root length, seedling shoot length and seedling dry weight (Mahakham *et al.,* 2017).

Seedling Vigor Indices

The data presented in Table 4 and 5 showed that the seeds of genotype EC 993950 had higher vigor indices, 3424 and 5268 as SVI-I and SVI-II than seeds of genotype JS 335 (3345 and 4892 as SVI-I and SVI-II) before ageing. However, after 72h of ageing the seeds of genotype JS 335 showed reduced vigor (1798 and 2285 as SV-I and SV-II) than EC 993950 (2321 and 3252 as SVI-I and SVI-II). A similar trend was observed in mean seedling length (Table 2) and mean seedling dry weight (Table 3) though the viability was not affected. The difference in vigor of seeds before ageing might be due to the difference in germination per cent, as the vigor estimation includes germination per cent for calculation. The effect of deterioration in seeds reflects as varied germination per cent in fresh and aged seeds. The reason for contrasting results in vigor after ageing between JS 335 and EC 993950 could be due to the deterioration and resistance exhibited in JS 335 and EC 993950, respectively. This shows that the genotype, EC 993950 with black colored seed coat has higher vigor after ageing than JS 335 with white seedcoat. Studies have already reported low vigor due to increased deterioration and damage in genotypes with colorless seed coat than in colored genotypes which have been related to the seed coat composition and thickness (Kazim, 2010).

When, the aged seeds were subjected to different priming treatments there was an increase in SVI-I and SVI-II in all the genotypes. Among priming treatments, increased SVI-I (2491 and 3007) and SVI-II (3225 and 4355) were recorded in the seeds of osmo priming-PEG at 1.1MPa for 6 hrs, followed by osmo priming-PEG at 1.1MPa for 12 hrs (SVI-I, 2311 and 2797) and SVI-II, 3061 and 4130) in white seed coat (JS 335) and black seed coat (EC 993950) genotypes, respectively (Table 4 and 5). The higher germination, mean seedling length and dry weight from invigorated seeds resulted in high seedling vigor indices (SVI I, SVI II). The increased food reserve mobilization efficiency of primed seeds resulted in better seed germination and seedling length which in turn increased the seedling vigor index I and II (Dey *et al*., 2013).

Table 4

Influence of ageing and priming treatments on SVI-I

	EC993608 (G1)	EC993850 (G2)	EC993950 (G3)	EC993703 (G4)	EC993937 (G5)	JS 335 (G6)	Mean
Fresh/control (T1)	3072	3111	3424	3188	3247	3345	3231
Ageing for 72 hrs (T2)	2092	2151	2321	1657	1716	1798	1956
Hydro priming (T3)	2211	2357	2492	1856	1928	2022	2145
Vit. C priming (T4)	2401	2552	2642	1996	2069	2186	2308
PEG for12 hrs (T5)	2589	2719	2797	2125	2200	2311	2457
PEG for 6 hrs (T6)	2783	2922	3007	2287	2396	2491	2648
MEAN	2525	2635	2781	2185	2259	2359	
	SEM	SED	CD				
TRT	11.94	16.89	44.69				
CONC	11.94	16.89	44.69				
T*G	29.26	41.37	109.47				
CV	2.06						

Table 5

Influence of ageing and priming treatments on SVI-II

	EC993608 (G1)	EC993850 (G2)	EC993950 (G3)	EC993703 (G4)	EC993937 (G5)	JS 335 (G6)	Mean
Fresh/control (T1)	4988	5125	5268	4550	4699	4892	4920
Ageing for 72 hrs (T2)	2938	2991	3252	1955	2100	2285	2587
Hydro priming (T3)	3409	3567	3714	2437	2507	2656	3048
Vit. C priming (T4)	3569	3743	4011	2586	2682	2942	3256
PEG for12 hrs (T5)	3658	3816	4130	2610	2772	3061	3341
PEG for 6 hrs (T6)	3828	3905	4355	2741	2888	3225	3490
MEAN	3732	3858	4122	2813	2941	3177	
	SEM	SED	CD				
TRT	17.47	24.70	65.36				
CONC	17.47	24.70	65.36				
T*G	42.79	60.51	160.10				
CV	2.15						

Electrical Conductivity of Seed Leachates

To confirm the differential response of genotypes with respect to deterioration of seeds due to ageing, the damage to the seed coat after accelerated ageing was measured as Electrical conductivity (EC) of seed leachates. The EC increased in all the genotypes with ageing period and there was a difference in EC values among genotypes. Results showed that, the fresh seeds had EC values of 15.11 $\mu Scm^{-1}g^{-1}$ and 11.57 $\mu Scm^{-1}g^{-1}$ and after 72 h of ageing it increased to 52.32 $\mu Scm^{-1}g^{-1}$ and 42.62 $\mu Scm^{-1}g^{-1}$ in EC 993950 and JS 335, respectively. But the genotype, EC 993950 showing comparatively lower EC levels than the genotype JS 335. When the aged seeds are subjected to different priming treatments the EC values were 33.03 $\mu Scm^{-1}g^{-1}$ and 28.62 $\mu Scm^{-1}g^{-1}$ in osmo priming-PEG at 1.1MPa for 6 hrs, followed by osmo priming-PEG at 1.1MPa for 12 hrs (34.80 $\mu Scm^{-1}g^{-1}$ and 30.69 $\mu Scm^{-1}g^{-1}$) in white seed coat (JS 335) and black seed coat (EC 993950) genotypes, respectively (Table 6).

ANOVA was used to test the significance of the data and there was a significant difference ($P<0.01$) in seed quality parameters between genotypes and between different ageing treatments. Electrical conductivity results showed that EC values increased as seeds aged. Parrish *et al.* (1982) reported that an increase in electrolyte leakage in deteriorated seeds is an indication of membrane deterioration leading to the ageing of seeds. However, the genotype, EC 993950 had comparatively lower EC than the genotype, JS 335 though both genotypes showed increased EC with ageing period. This shows that all the genotypes had different levels of membrane damage during ageing, with black genotypes having lower seed coat damage compared to white. The reason for the higher integrity of seed coat in black genotypes could be attributed to the presence of proanthocyanidins with free radical scavenging activity (Takahata *et al.,* 2001) which would in turn help in cell repair mechanisms preventing membrane damage (Bailly, 2004).

Total Dehydrogenase (TDH)

Results showed that the fresh seeds had comparatively higher TDH (1.461 OD @ A480 nm and 1.828 OD @ A480 nm) than the seeds subjected to ageing (0.794 OD @ A480 nm and 1.150 OD @ A480 nm) in white (JS 335) and black (EC 993950) genotypes, respectively. However, priming of the seeds increased the TDH activity. Among different priming treatments, the osmo priming-PEG at 1.1MPa for 6 hrs recorded higher TDH (1.181 OD @ A480 nm and 1.541 OD @ A480 nm), followed by osmo priming-PEG at 1.1MPa

Table 6

Influence of aging and priming treatments on electrical conductivity

	EC993608 (G1)	EC993850 (G2)	EC993950 (G3)	EC993703 (G4)	EC993937 (G5)	JS 335 (G6)	Mean
Fresh/control (T1)	13.18	11.93	11.57	17.00	16.49	15.11	14.21
Aging for 72 hrs (T2)	45.86	43.44	42.62	55.09	54.95	52.32	49.05
Hydro priming (T3)	36.09	35.41	34.64	42.26	41.91	39.35	38.28
Vit. C priming (T4)	34.58	33.16	32.84	40.36	40.00	37.22	36.36
PEG for12 hrs (T5)	33.05	31.37	30.69	37.75	37.40	34.80	34.18
PEG for 6 hrs (T6)	30.65	29.38	28.62	36.09	35.04	33.03	32.14
MEAN	32.24	30.78	30.16	38.09	37.63	35.30	
	SEM	SED	CD				
TRT	0.326	0.461	1.220				
CONC	0.326	0.461	1.220				
T*G	0.798	1.129	2.987				
CV	4.06						

for 12 hrs (1.100 OD @ A480 nm and 1.503 OD @ A480 nm) in white seed coat (JS 335) and black seed coat (EC 993950) genotypes, respectively (Table 7). Thus, the seeds of genotype JS 335 due to ageing and related deterioration had higher reduction in TDH than the genotype EC 993950. Previous studies have show that the TDH activity decreases in aged and deteriorated seeds (Verma *et al.,* 2003). The activity of TDH correlates with vigor and germination per cent of seeds. The seeds of genotype, JS 335 showed higher reduction in germination per cent, vigor and TDH that the genotype, EC 993950 with lower change in TD Hactivity. Dehydrogenase activity is an indicator of root vitality and used as comprehensive assessment

Table 7

Influence of ageing and priming treatments on TDH (OD @480 nm)

	EC993608 (G1)	EC993850 (G2)	EC993950 (G3)	EC993703 (G4)	EC993937 (G5)	JS 335 (G6)	Mean
Fresh/control (T1)	1.676	1.696	1.828	1.387	1.444	1.461	1.582
Ageing for 72 hrs (T2)	0.996	1.036	1.150	0.713	0.764	0.794	0.909
Hydro priming (T3)	1.237	1.270	1.390	0.952	1.004	1.031	1.147
Vit. C priming (T4)	1.258	1.291	1.309	0.972	1.025	1.094	1.158
PEG for12 hrs (T5)	1.279	1.364	1.503	0.993	1.117	1.100	1.226
PEG for 6 hrs (T6)	1.341	1.389	1.541	1.149	1.148	1.181	1.361
MEAN	1.354	1.392	1.501	1.067	1.133	1.157	
	SEM	SED	CD				
TRT	0.012	0.017	0.045				
CONC	0.012	0.017	0.045				
T*G	0.030	0.042	0.110				
CV	4.03						

TABLE 8

Interaction effect of treatments and genotypes on germination, mean seedling length, SVI-I, SVI-II, EC, TDH activity in fresh, aged and primed seeds

Treatment *Genotype	Germination %	Mean seedling length (cm)	SVI-I	SVI-II	EC ($\mu Scm^{-1}g^{-1}$)	TDH (OD @ A480 nm)
T1G1	88.67	34.65	3072	4988	13.18	1.676
T1G2	89.67	34.70	3111	5125	11.93	1.696
T1G3	90.33	37.90	3424	5268	11.57	1.828
T1G4	94.33	33.80	3188	4550	17.00	1.387
T1G5	94.67	34.30	3247	4699	16.49	1.444
T1G6	95.67	34.97	3345	4892	15.11	1.461
T2G1	73.00	28.66	2092	2938	45.86	0.996
T2G2	73.33	29.33	2151	2991	43.44	1.036
T2G3	75.00	30.95	2321	3252	42.62	1.150
T2G4	63.33	26.16	1657	1955	55.09	0.713
T2G5	63.67	26.95	1716	2100	54.95	0.764
T2G6	64.67	27.80	1798	2285	52.32	0.794
T3G1	75.33	29.35	2211	3409	36.09	1.237
T3G2	76.67	30.74	2357	3567	35.41	1.270
T3G3	77.67	32.09	2492	3714	34.64	1.390
T3G4	65.67	28.27	1856	2437	42.26	0.952
T3G5	66.00	29.22	1928	2507	41.91	1.004
T3G6	67.00	30.18	2022	2656	39.35	1.031
T4G1	76.00	31.59	2401	3569	34.58	1.258
T4G2	77.33	32.99	2552	3743	33.16	1.291
T4G3	78.00	33.87	2642	4011	32.84	1.309
T4G4	66.33	30.09	1996	2586	40.36	0.972
T4G5	67.00	30.89	2069	2682	40.00	1.025
T4G6	68.33	31.99	2186	2942	37.22	1.094
T5G1	77.00	33.63	2589	3658	33.05	1.279
T5G2	77.67	35.00	2719	3816	31.37	1.364
T5G3	78.33	35.70	2797	4130	30.69	1.503
T5G4	66.67	31.87	2125	2610	37.75	0.993
T5G5	67.33	32.68	2200	2772	37.40	1.117
T5G6	69.00	33.50	2311	3061	34.80	1.100
T6G1	78.33	35.53	2783	3828	30.65	1.676
T6G2	79.00	36.98	2922	3905	29.38	1.696
T6G3	80.00	37.59	3007	4355	28.62	1.828
T6G4	67.67	33.79	2287	2741	36.09	1.387
T6G5	68.67	34.90	2396	2888	35.04	1.444
T6G6	70.00	35.59	2491	3225	33.03	1.461
TOTAL	2707.33	1168.22	88463	123858	1225.25	45.627
MEAN	75.20	32.45	2457	3440	34.03	1.267

index reflecting the metabolic activity level and root's ability to absorb nutrients and water. The enhanced source to sink relation leads to better accumulation of food reserves like protein and carbohydrates (Mahakham *et al.,* 2017). Higher the dehydrogenase activity higher is the germination (Mahakham *et al.,* 2017).

Results in the present study showed that the genotypes with white seed coat are more susceptible to deterioration than genotypes with black seed coat. The reason for the higher resistance of seed coat in black genotypes could be attributed to the presence of lignin, tannin and other metabolites with free radical scavenging activity which would in turn help in cell repair mechanisms preventing membrane damage (Bailly, 2004)

Among different priming treatments imposed to the aged seeds, the osmo priming-PEG at 1.2MPa for 6 hrs recorded higher germination percentage, mean seedling length, SVI-I and SVI-II, lower EC and TDH in EC 993950 (black seed coat). The genotype, JS 335 recorded higher seed quality parameters among the genotypes with white seed coat with osmo priming-PEG at 1.1MPa for 6 hrs followed by osmo priming-PEG at 1.1MPa for 12 hrs. Primed seeds produced healthy and vigorous seedlings with increased gibberellin, hydrolytic enzyme production which aids in reserve mobilization which decides healthy seedlings (Zhu *et al*., 2010 and Wang *et al*., 2011).

The study explored how priming influences accelerated ageing in two contrast colored soybean seed coat genotypes, black and white, focusing on their resistance to deterioration. The genotype with black seed coat demonstrated greater resilience to ageing, as reflected in superior seed quality traits such as germination rate, root and shoot length, average seedling length and dry weight, as well as seedling vigor indices I and II. Membrane damage evaluated by measuring the electrical conductivity (EC) was higher in aged seeds compared to fresh seeds, indicating increased membrane permeability. However, seed priming particularly osmo priming with polyethylene glycol (PEG) significantly reduced EC, suggesting improved membrane stability. Dehydrogenase (TDH) activity, a marker of seed metabolic health was higher in fresh seeds and declined with ageing. Priming treatments enhanced TDH activity in aged seeds, with the most pronounced effect again observed in PEG-primed seeds treated for 6 hours. Seeds with white seed coat were more vulnerable to deterioration than those with black seed coat. The enhanced seed quality, lower EC and higher TDH activity in black-coated seeds point to better membrane integrity. This advantage is likely due to the presence of proanthocyanidins in the black seed coat, compounds known for their antioxidant properties that help mitigate cellular damage during aging.

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Unveiling the Pathogenic Potential of *Enterobacter* spp. in Bacterial Wilt of Solanaceous Vegetables in Karnataka, India

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Abstract

Bacterial wilt is a significant threat to solanaceous crops in India, traditionally attributed to *Ralstonia solanacearum*. However, emerging evidence suggests a broad range of pathogens may be involved. This study reports the isolation and characterization of four *Enterobacter* spp. strains (Ent-7, Ent-17, Ent-20 and Ent-32) from wilted tomato and brinjal plants collected from Bengaluru Urban, Tumkur, Hassan and Dharwad districts of Karnataka. Initial identification was based on colony morphology on triphenyl tetrazolium chloride (TTC) and nutrient agar, followed by biochemical and gram reaction. All isolates were Gram-negative, catalase-positive, oxidase-negative rods consistent with *Enterobacter* spp. Molecular confirmation using 16S rRNA gene sequencing revealed that two isolates (Ent-7, Ent-17) were *Enterobacter bugandensis*, while Ent-20 and Ent-32 were identified as *E. chuandaensis* and *E. mori*, respectively. Pathogenicity assays under controlled conditions showed that all four isolates could induce wilt-like symptoms in their respective host plants, albeit with slower symptom development and lower aggressiveness compared to *R. solanacearum*. Re-isolation of the same strains from symptomatic tissues confirmed Koch's postulates. This is the first of its kind from Karnataka, demonstrating the wilt-causing potential of *Enterobacter* spp. in solanaceous crops. The findings suggest that these bacteria may act as emerging opportunistic pathogens in bacterial wilt complexes, especially under favorable environmental conditions or in compromised hosts. These results underscore the need to consider non-*Ralstonia* bacteria in wilt diagnostics and to revise integrated disease management approaches accordingly.

Keywords : *Enterobacter*, Bacterial wilt, Solanaceous crops, *Ralstonia solanacearum*, Karnataka

Tomato (*Solanum lycopersicum L.*) and brinjal (*Solanum melongena L.*) are major solanaceous crops of global economic significance, serving as critical components of food and nutritional security. India is one of the leading producers of both tomato and brinjal, with tomato cultivated on approximately 1.15 million hectares, yielding 20.4 million tonnes, and potato on 2.65 million hectares, producing over 56 million tonnes during 2021-22 (Ministry of Agriculture and Farmers Welfare, 2022). These crops form a vital part of India's horticultural economy, particularly in states such as Karnataka, West Bengal, Uttar Pradesh and Bihar. However, their productivity is frequently hampered by soil-borne bacterial pathogens, of which bacterial wilt remains one of the most devastating and difficult-to-manage diseases (Bhanwar *et al*., 2021).

Tomato and brinjal are susceptible to a wide array of fungal, bacterial, and viral diseases that collectively reduce crop productivity. Among the major fungal

pathogens, early blight (*Alternaria solani*), Fusarium wilt (*Fusarium oxysporum*) and Phomopsis blight (*Phomopsis vexans*) are frequently reported and economically damaging (Shete *et al.*, 2021 and Kumar *et al.*, 2018). Bacterial diseases such as bacterial wilt (*Ralstonia solanacearum*), bacterial spot (*Xanthomonas* spp.), bacterial speck (*Pseudomonas syringae*) and soft rot (*Pectobacterium* spp.) pose significant threats during both field production and post-harvest storage (Choudhary *et al.*, 2018 and Kennelly *et al.*, 2007). Viral infections such as tomato leaf curl virus (ToLCV) is alsoincreasing, often causing severe yield losses, (Achari *et al*., 2019). This diverse and complex disease scenario emphasizes the need for integrated pathogen monitoring and effective management practices.

Traditionally, bacterial wilt in solanaceous crops has been predominantly attributed to the *Ralstonia solanacearum* species complex (RSSC), a vascular pathogen known for its broad host range and genetic diversity (Hayward, 1991 and Genin & Denny, 2012). Nevertheless, recent studies have highlighted the emergence of other bacterial genera as potential wilt pathogens. Notably, many species of the genus *Enterobacte* have been increasingly implicated in vascular wilt symptoms in tomato and potato (Kaushik *et al.*, 2022). These Gram-negative, facultatively anaerobic rods are often environmental or opportunistic bacteria, but their role as primary phytopathogens is gaining attention due to increasing reports of their isolation from symptomatic plant tissues.

Chemical control of bacterial wilt has shown limited success due to the soil-borne and systemic nature of the pathogen, as well as the persistence of bacterial populations in alternate hosts and infected crop residues (Yuliar *et al.*, 2015 and Pradhan *et al.*, 2020). Therefore, the identification of pathogen diversity and virulence factors remains essential for developing effective integrated disease management strategies, including host resistance breeding. Despite growing reports on the involvement of *Enterobacter* spp. in wilt disease, detailed pathogenicity studies, molecular characterization and virulence profiling remain limited, particularly in Indian contexts.

The pathogenic potential of *Enterobacter* spp. in solanaceous crops represents a significant shift in the known etiology of bacterial wilt and may have profound implications for diagnostic protocols and management practices (Sarkar & Chaudhuri, 2015 and Ning *et al*., 2024). Their emergence may be attributed to agroecological changes, crop intensification or overlooked pathogenicity due to symptom similarity with RSSC infections. Moreover, limited molecular and pathogenicity data exist to support their definitive role as causative agents under field conditions in India.

The study investigated bacterial wilt outbreaks in tomato and potato fields across selected districts of Karnataka. A systematic polyphasic approach was employed for the isolation and characterization of the pathogens. Isolates exhibiting wilt symptoms were subjected to Gram staining, biochemical profiling and pathogenicity assays to establish their phenotypic identity and confirm Koch's postulates. Furthermore, molecular confirmation was carried out using PCR amplification and sequencing of conserved regions such as 16S rRNA, which enabled accurate species-level identification. The detection of *Enterobacter spp.* as wilt-causing organisms represents a novel finding in the context of Indian agriculture.

This study aims to elucidate the pathogenic profile and confirm the etiological role of these *Enterobacter* species in bacterial wilt of tomato and potato. The findings contribute to a growing body of literature that expands our understanding of emerging plant pathogens and highlight the importance of integrating both classical and molecular tools for accurate pathogen diagnostics and sustainable disease management.

MATERIAL AND METHODS

Collection of Bacterial Wilt Samples

A field survey was conducted during the 2023-2024 cropping season in Karnataka, India, to identify

bacterial wilt symptoms in key solanaceous vegetable crops. The crops examined were tomato, potato, chili and brinjal. Symptomatic plants were sampled from farmers' fields located across different districts of Karnataka. Wilted plants were carefully uprooted and surface contaminants such as soil particles and debris were removed at the site of collection. Samples were immediately placed in sterile polythene bags, appropriately labelled with location and date. The collected samples were transported to the laboratory for pathogen isolation and further analysis. Details of the sampling sites and corresponding isolate information are presented in Fig. 1 and Table 1.

Isolation of the Pathogen and Storage

For the isolation of the pathogen, vascular tissue was cut into small pieces measuring 4-5 mm in length.

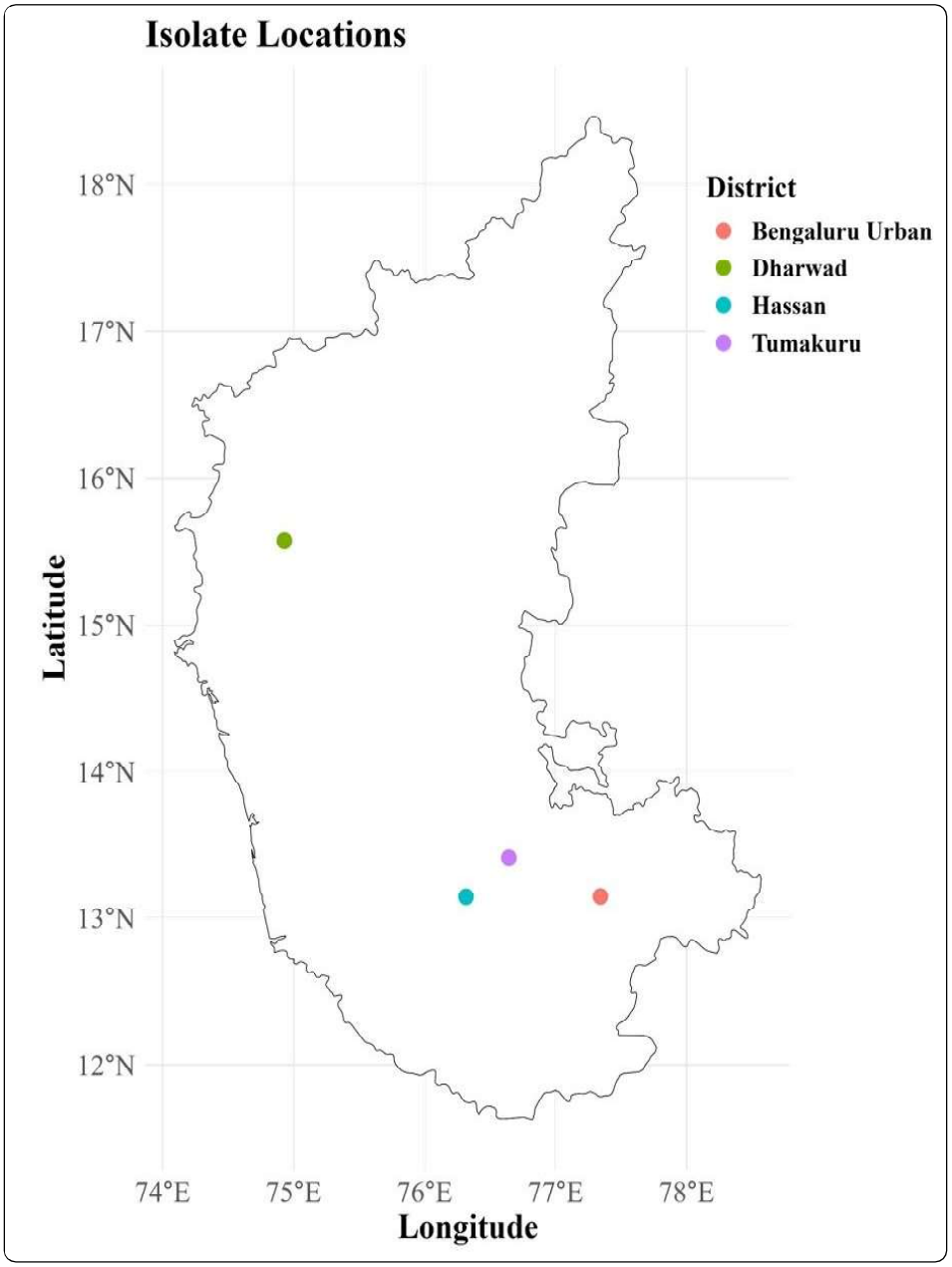

Fig. 1 : Karnataka map showing the list of *Enterobacter* spp.isolates used for the study

Table 1

List of *Enterobacter* spp. isolates used for the study

District	Taluk	Village	Coordinates	Crop	isolate
Bengaluru Urban	Yelahanka GKVK	Horticulture dep.	13.1485°N/77.3426°E	Tomato	Ent-7
Tumakuru	Tiptur	Agrahara	13.3124°N/76.3439°E	Brinjal	Ent-17
Hassan	Belur	Kanakenahalli	13.1465°N/76.5165°E	Brinjal	Ent-20
Dharwad	Dharwad	Garaga	15.5700°N/74.9296°E	Tomato	Ent-32

The tissue was surface-sterilised by immersing it in a 0.1 per cent sodium hypochlorite solution for 30 seconds. The disinfected tissue was washed in sterile water to remove any traces of sodium hypochlorite. The surface-sterilised bits were then suspended in 10 mL of sterile distilled water in a test tube for 10 minutes. After the water in the test tube became turbid due to oozing of the bacterial cells from the cut ends of the diseased tissue the bacterial suspension was serially diluted in nine millilitres of sterile distilled water Kiseleva *et al*., 2021).

One hundred microliters of the diluted bacterial suspension was poured onto the surface of solidified triphenyl tetrazolium chloride agar (TZC) medium (Kelman,1954) in sterilized glass petri plates. The bacterial suspension was spread evenly onto the surface of TZC medium using a sterilized spreader. The inoculated plates were incubated at 32°C for 48 hours. At the end of the incubation period, the plates were observed for the development of colonies.

The well-separated virulent colonies of *Enterobacter* spp. were picked up with a sterile inoculation loop and suspended in sterile distilled water in sterile propylene culture tubes. They were stored at 4°C in a refrigerator for further use as a stock culture. Additionally, well-separated, typical virulent colonies of *Enterobacter* spp. were picked with a sterile inoculation loop and suspended in 100 mL of Nutrient broth. The suspension was then incubated at 32°C for 36 hours in a rotary shaker at 160 rpm. About 800 µL of this overnight bacterial culture of *Enterobacter* spp. was pipetted into sterile microfuge tubes and 800 µL of 80 per cent auto claved glycerol was added and the culture tubes were then vortexed. The bacterial culture was then frozen in liquid nitrogen and stored at -80°C for further study.

Cultural and Biochemical Characters

The presumptive identification of the pathogen was initially based on colony morphology observed on TZC medium. Key features including colony shape, size, pigmentation, margin regularity and mucoid appearance were recorded after 48 hours of incubation at 32 °C. Colonies suspected to belong to *Enterobacter* spp. were typically round, smooth, raised and exhibited a glistening, mucoid texture with off-white pigmentation, consistent with previous descriptions (Wu *et al.*, 2020b).

Gram Staining

A loop full of bacterial culture was smeared on the clean glass slide and was air dried. Further, it was exposed to flame for two to three times for a very short time and then covered with crystal violet for 1 minute. The slide was washed with distilled water and covered with iodine solution for 1 minute. The iodine solution was washed with 95 per cent ethyl alcohol and subsequently with distilled water, drained and counterstained and safranin was applied for 1 minute (Gerhardt *et al.,* 1981). The slide was washed with distilled water, dried with tissue paper, air dried and then subjected to microscopic examination under an oil immersion objective lens.

Catalase Test

Catalase is an enzyme that decomposes hydrogen peroxide into water and oxygen. A loopful of 48 hrs slant growth of the test bacterium was smeared on a slide and covered with a few drops of hydrogen peroxide (20 volumes). The reaction will be positive if gas bubbles are produced. The production of gas bubbles provides a clue to the presence of aerobic and facultative anaerobic bacteria (Taylor and Achanzar, 1972).

Oxidase Test

The oxidase reaction was performed using commercially available oxidase discs (DD018, HiMedia, Mumbai). A well-isolated colony was picked using a sterile inoculation loop and rubbed onto the disc surface. The reaction was observed within 5-10 seconds at a temperature of 25-30 °C. Development of a purple color within 10 seconds was recorded as oxidase-positive, color change occurring between 10-60 seconds was considered delayed positive and no color change after 60 seconds was interpreted as oxidase-negative (Gaby and Hadley, 1957).

KOH Solubility Test

A loopful of 48 hours slant growth of the test bacterium was smeared on a slide and 2-3 drops of 3 per cent KOH was added. The colony of the test strain was stirred into the solution with a clean loop for 5 to 10 seconds. When the solution was viscous enough to stick to the loop, causing a thin strand of slime or thread, the test was recorded as positive (KOH soluble). The KOH test was used as a complementary method to confirm Gram reaction, following the procedure described by Suslow *et al.* (1982).

Pathogenicity Test

Twenty-day-old seedlings of tomato and brinjal were uprooted and the roots were washed in water and a few tertiary roots were sheared with sterilized scissors. The injured roots of the seedlings were dipped in the bacterial suspension (OD at A_{600}sm=0.6, 10^8 CFU/ ml) separately for each isolate of *Enterobacter* spp. for ten minutes. Then they were transplanted to plastic bags containing sterilized soil. Plants similarly inoculated with sterile water served as negative controls. The nutrients required for plant growth were supplied through a plant nutrient solution at regular intervals. The plants were watered regularly and observations for the appearance of wilt symptoms were recorded. The plants expressing wilt symptoms were selected and the bacterium was re-isolated and compared with the original culture of *Enterobacter* spp. to satisfy Koch's postulates and confirm causative agent identity, following protocols similar to other enterobacterial wilt studies (Jeevan *et al*., 2022 and Ning *et al*., 2024).

Isolation of Genomic DNA from *Enterobacter* spp.

The genomic DNA of all 4 isolates was isolated following the procedure given by Patel *et al*. (2023) with slight modifications. The genomic DNA extraction protocol involves the following steps: (a) 2.0 mL of the 24 h old bacterial culture was taken in an Eppendorf tube and centrifuged at 10,000 rpm for 10 min at 4 °C, (b) The supernatant was discarded and the pellet was washed with 1.5 mL of sterile distilled water, (c) Pellet re-suspended in 600 µL of genomic DNA lysis buffer which consists of 564 µL 1X Tris HCl-EDTA buffer, 30 µL of 10 per cent Sodium dodecyl sulfate (SDS) and 6 µL of Proteinase K (10 mg/mL), (d) The tube is incubated at 65 °C for 15-20 min, (e) 200 µL of 5 M NaCl and 120 µL cetrimonium bromide (CTAB) previously heated at 65 °C was added to the mixture, incubated at 65 °C for 15 min, (f) An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added, (g) Tube was mixed well and centrifuged at 10,000 rpm for 10 min at room temperature, (h) Aqueous phase was transferred to a new Eppendorf tube, (i) DNA was precipitated with an equal volume of chloroform: isopropyl alcohol (24:1), centrifuged at 10,000 rpm for 10 min at room temperature, (j) The supernatant was transferred to a new Eppendorf tube, (k) 0.6 times the volume of the supernatant obtained, iso-propanol and 50 µL of sodium acetate was added and mixed to see a white DNA precipitate which is

incubated for overnight at -20 °C, (l) Centrifuged at 10,000 rpm for 10 min at room temperature to obtain the DNA pellet, (m) DNA pellet then washed twice with 70 per cent ethanol, air dried for 30 min, (n) Pellet was dissolved in 50 µL TE Buffer (10 mM Tris, 1 mM EDTA, pH 8), followed by treatment with 2 µL of RNase and incubated at 37 °C for 30 min and (o) DNA samples are stored at 20 °C.

Qualitative Analysis of Genomic DNA

DNA concentration was measured using Nano Drop (De Novex) at 260 /280 nm. The quality of the DNA was checked on 0.8 per cent agarose gel in Tris-Acetate-EDTA (TAE) buffer (45 mM Tris-acetate, 1 mM EDTA, pH 8.0). The gel was then visualized in a Bio-Rad Imager.

Amplification and Sequencing of 16S rRNA Gene

For molecular diagnosis, an internal portion of the 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the universal primer set Fd1 (52 -AGAGTTTGATCCTGGCTCAG-32) and Rp1 (32 -ACGGCTACCTTGTTACGACTT-52). The reaction mixture consisted of 10/ µL of the 2X master mix, 100/ ng of genomic DNA as template, 1 µL each of forward and reverse primers (10 µM) and 7 µL of sterile molecular-grade distilled water. The components were mixed gently and briefly spun before placing them in a thermal cycler. The thermal cycling program for the amplification of the 16S rRNA gene consisted of initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. In master cycler (Eppendorf Hamburg, Germany). A 5µL aliquot of each amplification product was electrophoresed on a 1.2 per cent agarose gel in 1xTAE buffer at 50 V for 45 min, stained with ethidium bromide and the PCR products were visualized with a molecular imager (BIO-RAD, California, USA), the PCR product was outsourced for Sanger sequencing (Eurofins Scientific India Pvt. Ltd.).

BLAST Analysis, GenBank Deposition and Phylogenetic Analysis

Bacterial sequences were reverse complemented using BioEdit (https://www.bioinformatics.org/sms/rev_comp.html). Further DNA sequences were aligned using the Mega11 software. The aligned DNA sequences were compared in the National Center for Biotechnology Information (NCBI) database to identify bacterial homology with 16S rRNA sequences using the BLASTN tool with default parameters (http://blast.ncbi.nlm.nih.gov). The reference sequences required for comparison were downloaded from the NCBI database. Based on maximum query coverage, the bacterial species were identified. A phylogenetic tree was constructed using the 16S rRNA gene sequences of isolates. The sequences were submitted to GenBank to get accession numbers.

Pairwise genetic distance among the four *Enterobacter* isolates (Ent-7, Ent-17, Ent-20 and Ent-32) and selected reference strains was assessed using the 16S rRNA gene sequences previously obtained for phylogenetic tree construction. These sequences were input into the Sequence Demarcation Tool (SDT) version 1.3 software, which generated a color-coded heatmap matrix indicating percentage identity and clustering patterns based on conserved and divergent gene regions. The heatmap provided a comparative overview of evolutionary divergence among isolates and reference sequences.

Results and Discussion

Isolation and Morphological Characterization of *Enterobacter* Isolates

Four bacterial isolates, designated Ent 17, Ent 21, Ent 28 and Ent 32, were successfully isolated from vascular stem tissues of wilted tomato and brinjal plants collected from four major tomato-producing districts of Karnataka: Bengaluru Urban, Hassan, Tumkur and Dharwad. Although the primary objective was the isolation of *Ralstonia solanacearum*, four isolates of *Enterobacter* spp. were also obtained from wilted tomato and brinjal plants collected in

Bengaluru Urban, Hassan, Tumkur and Dharwad districts. These isolates were recovered from vascular tissues using standard bacteriological protocols. The isolates were initially cultured on TTC medium, where they developed smooth, round, light pink to pale red colonies characteristic of *Enterobacter* spp. (Fig. 2) (Wu *et al*. 2020b).

Fig. 2 : Growth of Ent-7 isolate on TZC medium

These colonies were then sub-cultured and purified on nutrient agar (NA) for further identification. All four isolates developed smooth, glistening, creamy white colonies with convex elevation and entire margins after 24-48 hours of incubation at 32 °C (Plate 2). These colony features are highly consistent with the characteristic appearance of *Enterobacter* spp., as described by Grimont and Grimont (2006). According to their taxonomy, *Enterobacter* colonies are typically mucoid, opaque, off-white to cream-colored and grow well on general-purpose media such as nutrient agar.

Such mucoid and glistening colony morphology is often attributed to extracellular polysaccharide (EPS) production, which contributes to biofilm formation and virulence, especially in vascular pathogens like *Enterobacter* and *Ralstonia* species (Wu *et al.*, 2020b). These traits may play a role in the ability of these isolates to colonize host xylem vessels and evade plant defenses, supporting their putative role in wilt development. The consistency in colony appearance across all isolates from distinct geographic regions further strengthens their identity as *Enterobacter* spp.. It supports the hypothesis that this genus may be emerging as a wilt-associated pathogen in solanaceous crops under Indian field conditions.

Biochemical Characterization of *Enterobacter* Isolates

To confirm the identity of the *Enterobacter* isolates (Ent-7, Ent-17, Ent-20 and Ent-32), standard microscopic and biochemical assays were conducted, including Gram staining, oxidase, catalase and KOH solubility tests. Each of these tests helped differentiate *Enterobacter* from other bacterial genera and support its preliminary identification. The results of these tests are given in Fig. 3 and Table 2.

All four isolates were found to be Gram-negative and short rod-shaped when observed under light

TABLE 2

Biochemical and cultural characteristics of *Enterobacter* spp. isolates

Isolate	Biochemical test			
	Gram staining	Oxidase	Catalase	KOH test
Ent-7	Negative	-	+	+
Ent-17	Negative	-	+	+
Ent-20	Negative	-	+	+
Ent-32	Negative	-	+	+

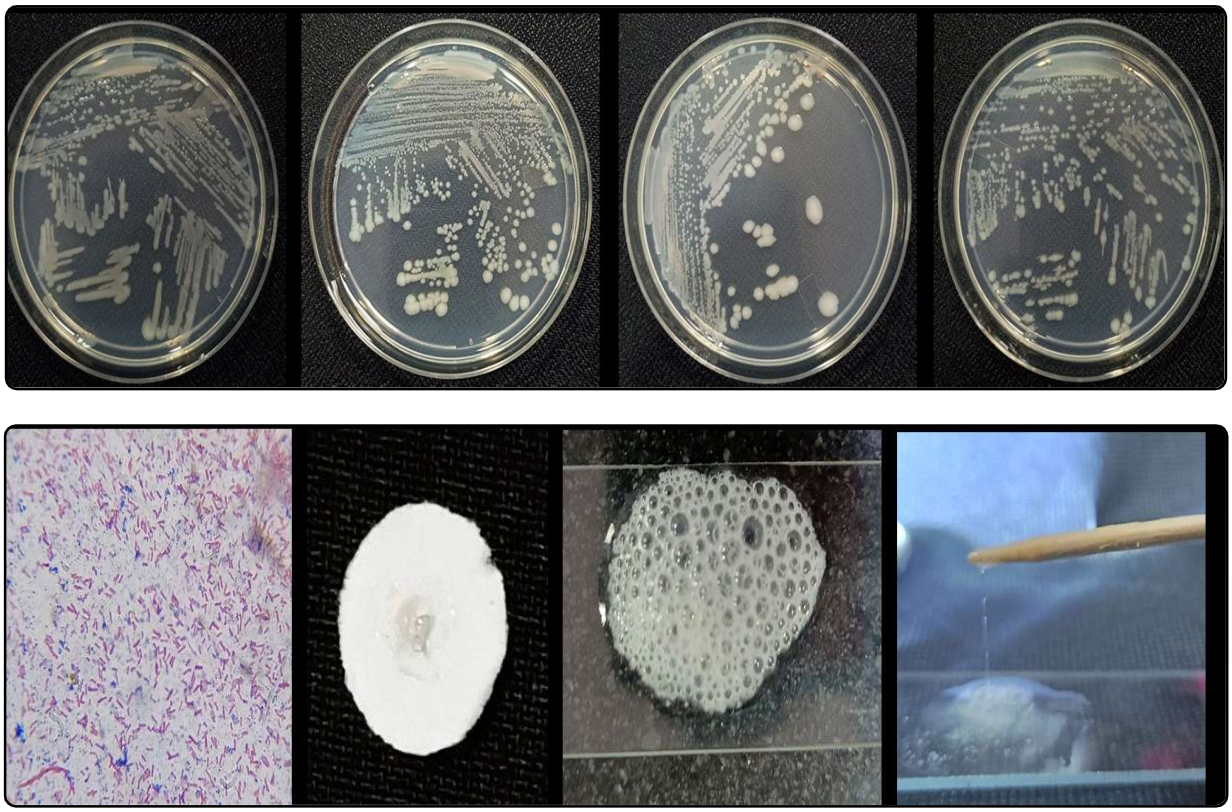

Fig. 3 : Cultural and biochemical characterization of *Enterobacter* spp. isolates. A) Colony characters of *Enterobacter* spp. isolates on NA medium, B) Gram negative, Oxidase negative, catalase positive and KOH test positive results

microscopy following Gram staining. These results indicate that the isolates possess a thin peptidogly can layer, allowing decolorization by alcohol and uptake of the counterstain, safranin, thus appearing pink under the microscope. This morphology is consistent with the Enterobacteriaceae family, which includes many rod-shaped, Gram-negative bacteria (Grimont and Grimont, 2006).

The oxidase test was negative for all four isolates, indicated by the absence of a color change on oxidase reagent strips. This confirms the lack of cytochrome oxidase, an enzyme involved in electron transport in oxidase-positive bacteria such as *Ralstonia* spp. This result is characteristic of *Enterobacter* spp., which are well known for being oxidase-negative due to their reliance on other terminal oxidases for respiration (Cappuccino and Sherman, 2014).

All isolates exhibited catalase-positive activity, evident from the immediate effervescence when hydrogen peroxide was applied. This confirms the presence of catalase enzyme, which detoxifies hydrogen peroxide into water and oxygena key trait for survival in aerobic environments. Catalase activity is common among aerobic and facultatively anaerobic bacteria, including *Enterobacter* spp. and *Ralstonia solanacearum*. (Taylor and Achanzar, 1972). This result further supports the isolates' ability to persist in oxygenated environments such as plant xylem vessels, where reactive oxygen species are part of the plant's defense response.

All isolates tested positive in the KOH solubility test, forming a viscous string when mixed with 3 per cent potassium hydroxide, confirming their Gram-negative nature. This reaction occurs due to the alkaline lysis of the outer membrane, which is characteristic of Gram-negative bacteria with relatively fragile cell

walls, leading to the release of viscous DNA (Suslow *et al*., 1982). In contrast, Gram-positive bacteria have a thicker, rigid peptidogly can layer that resists lysis.

The collective results-Gram-negative reaction, oxidase negativity, catalase positivity and positive KOH solubility-strongly support the identification of these isolates as *Enterobacter* spp. These characteristics closely align with descriptions by Grimont & Grimont (2006) and Wu *et al*. (2020b). Comparatively, *Xanthomonas oryzae* shares similar Gram and catalase profiles but differs in cell shape and pigmentation (Swathi *et al*., 2023), while *Ralstonia solanacearum* another vascular wilt pathogen, is oxidase-positive and exhibits different colony characteristics (Genin and Denny, 2012). Thus, these basic tests, while simple are valuable for distinguishing among morphologically similar Gram-negative phytopathogens.

Pathogenicity Test

All four *Enterobacter* isolates were tested for pathogenicity on their respective hosts under controlled conditions. Inoculated plants exhibited mild to moderate wilt symptoms, including partial leaf drooping, reduced turgor and stunted growth, typically emerging between 12- and 21-days post-inoculation (Fig. 4). Notably, symptom expression was slower and less severe than that caused by *Ralstonia solanacearum*, which typically leads to rapid and complete wilting. Most visible symptoms developed between 18 and 24 days, suggesting that while these *Enterobacter* isolates can cause wilt-like effects, their virulence is relatively low. Re-isolation from symptomatic tissues consistently yielded colonies matching the original cultural and morphological traits, thereby satisfying Koch's postulates and confirming the pathogenic potential of the isolates under favorable conditions.

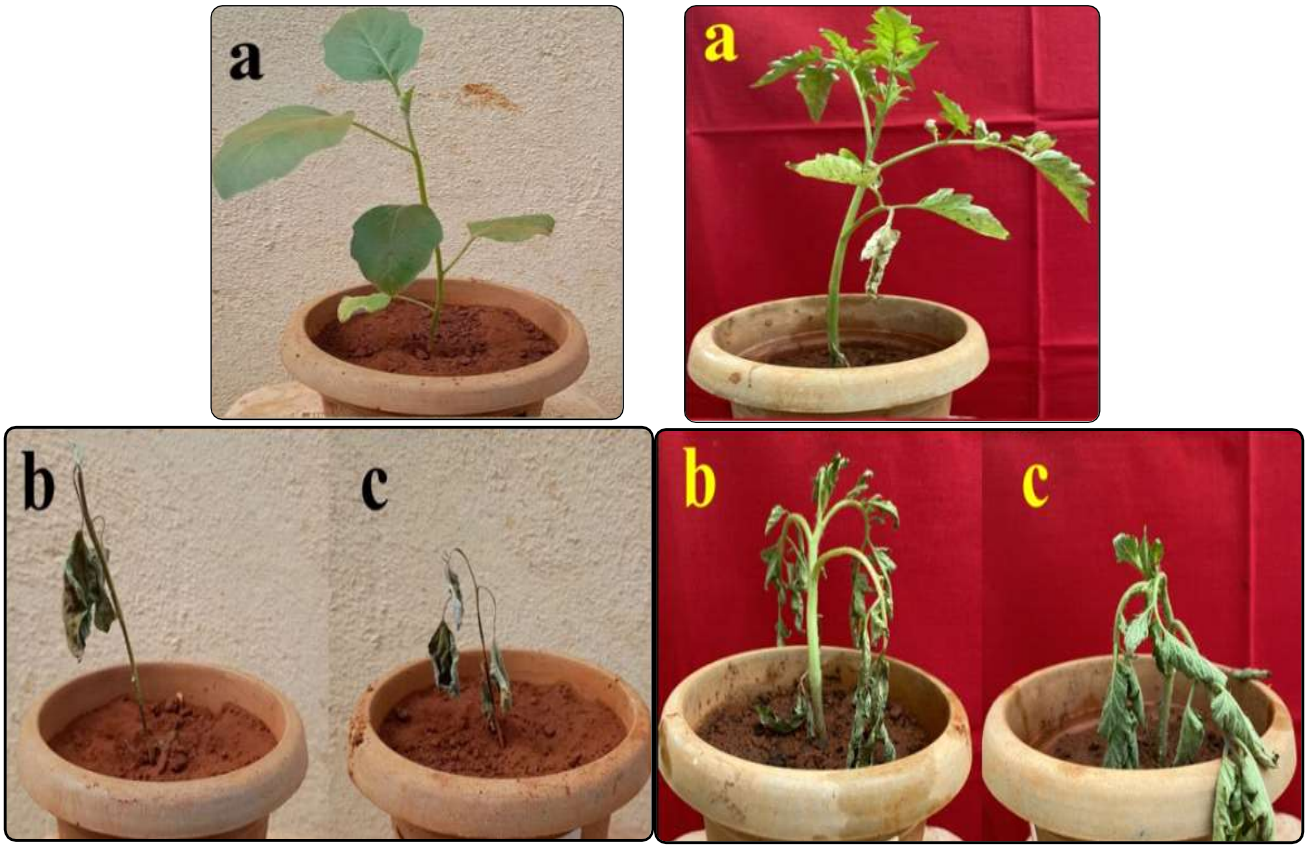

Fig. 4 : Pathogenicity test of *Enterobacter* spp. isolates. A) Brinjal isolates (a: Control, b: Ent-7, c: Ent-32), B) Tomato isolates (a: control, b: Ent-17, c: Ent-20)

This pattern supports the hypothesis that *Enterobacter* spp. may act as opportunistic or secondary pathogens, capable of colonizing vascular tissues and inducing wilt symptoms, particularly when host defenses are compromised. Similar findings were reported by Kämpfer *et al*. (2015), who documented wilt induction by *Enterobacter* in stressed plants and Ning *et al.* (2024), who confirmed *Enterobacter mori* as a causative agent of bacterial wilt in tomato under greenhouse conditions. Furthermore, recent studies by Patel *et al.* (2023) and Kathi (2024) suggest that some *Enterobacter* strains can express virulence factors such as cellulases and pectinases, enabling them to breach plant vasculature, though typically with lower aggressiveness compared to *Ralstonia solanacearum*.

Overall, the delayed symptom on set and partial wilting observed in this study underscore the importance of considering *Enterobacter* spp. in the context of bacterial wilt complexes. While they may not be primary pathogens, their interaction with other microbes or host stress conditions could amplify disease severity. These findings highlight the need for integrated pathogen monitoring, particularly under field scenarios involving multiple vascular bacteria.

Molecular Confirmation of *Enterobacter* spp. Isolates

Molecular characterization of the four *Enterobacter* isolates (Ent 17, Ent 21, Ent 28 and Ent 32) was performed using 16S rRNA gene amplification and sequencing. PCR amplification successfully yielded clear, single bands of approximately 1500 bp in all four isolates, as shown in Fig. 5. The absence of amplification in the no-template control confirmed the specificity and reliability of the PCR reaction. The ~1500 bp amplicon size corresponds to the expected length for bacterial 16S rRNA genes, indicating successful targeting of the conserved ribosomal region (Weisburg *et al*., 1991).

Sequencing and subsequent BLAST analysis revealed that the isolates shared high sequence identity (99.47-99.86%) with known *Enterobacter* species in the NCBI GenBank database.

Specifically, isolates Ent 7 and Ent 17 were identified as *Enterobacter bugandensis*, Ent 20 as *E. chuandaensis* and Ent 32 as *E. mori* (Table 3). These identifications were further supported by phylogenetic analysis, which grouped the isolates

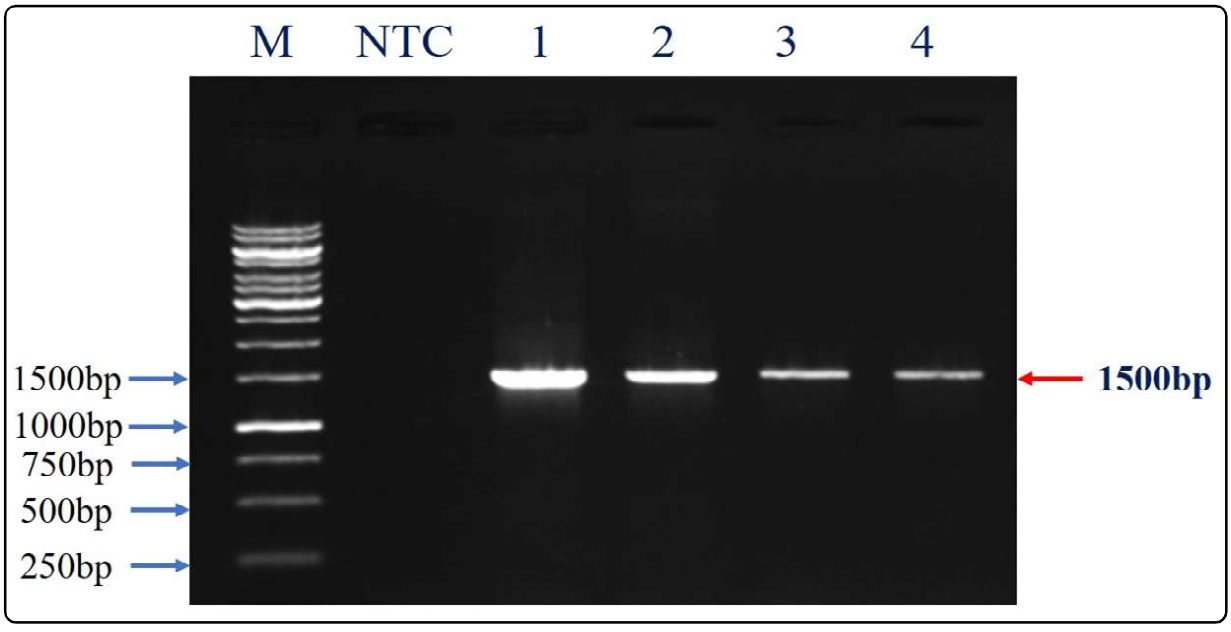

Fig. 5 : Molecular confirmation of *Enterobacter* spp. isolates using 16S rRNA amplification. L: 1 kb ladder, NTC: Non-template control, Lane 1-4: 1: Ent-7, 2: Ent-17, 3: Ent-20, 4: Ent-32

TABLE 3

Percentage identity and closest matches of *Enterobacter* isolates

Isolate	NCBI Accession numbers	Reference	Percentage similarity
Ent-7	PV300376	*Enterobacter bugandensis* (OQ891269)	99.86 %
Ent-17	PV300377	*Enterobacter bugandensis* (PQ781582)	99.58 %
Ent-20	PV300374	*Enterobacter chuandaensis* (MK049966)	99.93 %
		Enterobacter chuandaensis (OP355218)	99.86 %
Ent-32	PV300379	*Enterobacter mori* (OR752055)	99.72 %
		Enterobacter mori (OR752047)	99.47 %

within distinct, well-supported clades of their respective species (Fig. 6). Isolates Ent 7 and Ent 17 clustered closely with *E. bugandensis* strains such as MT103026 and PQ781582, while Ent 20 grouped with *E. chuandaensis* accessions like MT636544 and MK049966. Ent 32 was nested within the *E. mori*clade alongside strains such as OR752055 and OR752047.

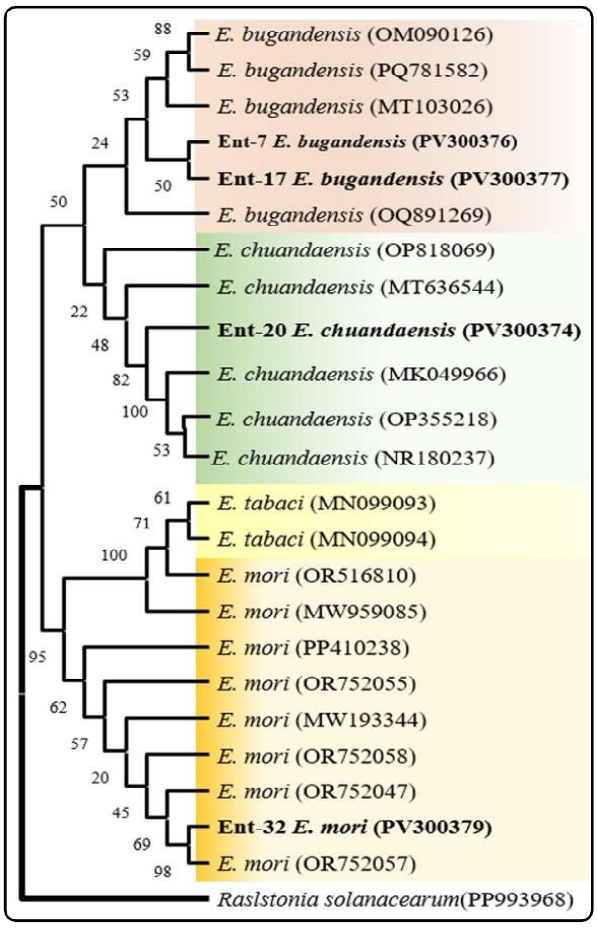

Fig. 6 : Phylogenetic tree constructed using MEGA XI analysis of 16S rRNA sequences of *Enterobacter* isolates

The 16S rRNA pairwise genetic distance heatmap (Fig. 7) illustrates that isolates Ent 7 and Ent 17 cluster most closely with *Enterobacter bugandensis* reference strains (dark red), whereas Ent 20 aligns with *E. chuandaensis* (orange-red) and Ent 32 groups with *E. mori* strains (yellow-red), reflecting 99.4-99.9 per cent sequence similarity. These patterns confirm BLAST and phylogenetic assignments and meet the ≥ 98.65 per cent similarity threshold conventionally used for species definition (Weisburg *et al*., 1991). However, given the limited discriminatory power of 16S rRNA alone, especially among closely related *Enterobacter* species, further resolution using housekeeping genes or MLSA is advisable (Kim *et al*., 2014).

Additionally, emerging evidence of plant-pathogenic *Enterobacter* species, including *E. asburiae* causing leaf blight in sorghum (Chen *et al*., 2023) and *E. cloacae* causing wilt in marigold (Jeevan *et al*., 2022), aligns well with our findings, underscoring the expanding role of *Enterobacter* in plant disease contexts.

The identification of *E. bugandensis,* recognised initially as a clinical pathogen (Patti *et al*., 2018) in wilted tomato and brinjal tissues, underscores its ecological flexibility and potential emergence as a plant-associated pathogen. In India, particularly in West Bengal, few studies have reported

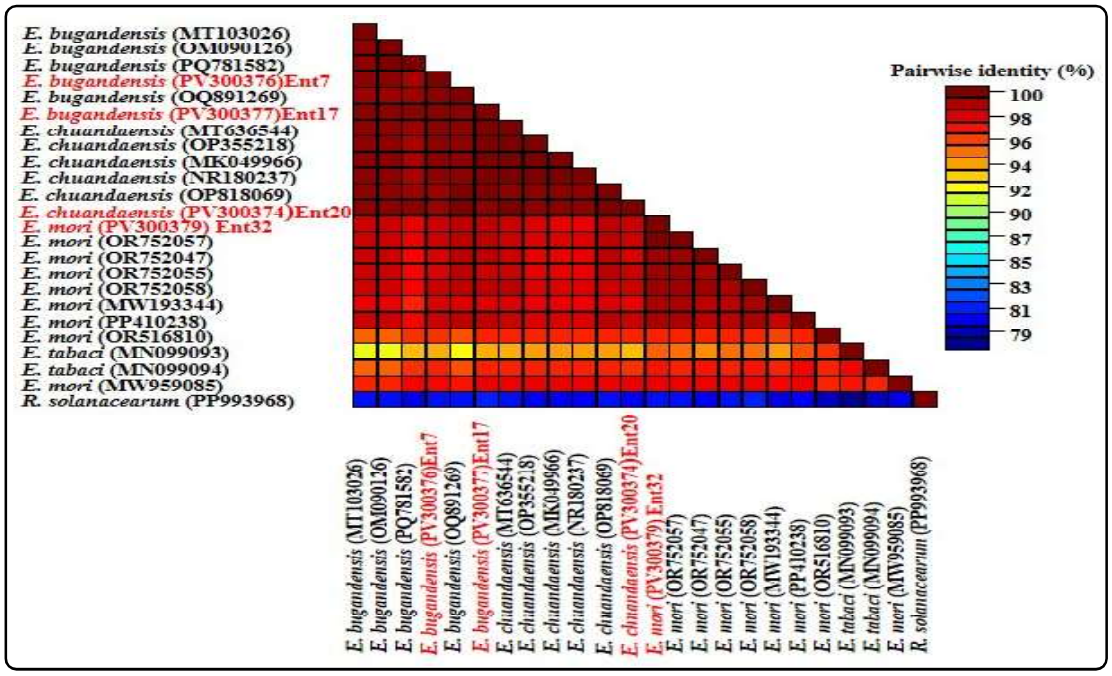

Fig. 7 : Heat map representation of pairwise genetic distance among *Enterobacter* isolates based on 16S rRNA sequences

Enterobacter spp. and other non-*Ralstonia* bacteria in wilted solanaceous crops. Sarkar and Chaudhuri (2015) reported *Enterobacter cowanii* and *Klebsiella oxytoca* as causal agents of bacterial wilt in tomato and chilli confirmed through molecular and biochemical methods. Similarly, Singh *et al*. (2015) documented the widespread occurrence of bacterial wilt in West Bengal. They highlighted the diversity of bacterial wilt pathogens beyond *R. solanacearum*, emphasizing the significance of Enterobacteriaceae in crop infections.

The detection of *E. chuandaensis*, a soil bacterium newly described by Wu *et al*. (2020a), from diseased plant tissues in this study suggests that its ecological range may be broader than previously thought. Likewise, *E. mori*, traditionally associated with silkworms, has now been increasingly isolated from environmental and plant-associated niches (Zhu *et al*., 2011). Its identification in wilted tomato and brinjal plants implies a possible role in plant pathogenesis or secondary colonization.

In conclusion, this study presents the first comprehensive report from Karnataka, India, demonstrating the pathogenic potential of *Enterobacter* spp. in causing bacterial wilt-like symptoms in tomato and brinjal. Through polyphasic characterization encompassing morphological, biochemical, pathogenicity and molecular analyses, four isolates were confirmed as *E. bugandensis*, *E. chuandaensis* and *E. mori*. Though these isolates induced milder and slower-developing wilt symptoms compared to *Ralstonia solanacearum*, their consistent recovery and virulence under controlled conditions fulfil Koch's postulates and indicate their emerging role as opportunistic or secondary pathogens in vascular wilt complexes. These findings not only expand the known pathogenic spectrum of *Enterobacter* spp. but also underscore the importance of revisiting wilt diagnostics and integrated management strategies under Indian field conditions, especially in regions like Karnataka where solanaceous crops form a critical part of the agro-economy.

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Evolutionary Relationship among the *Impatiens* of Karnataka, India : Inference from ITS Region Sequence Analysis

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ABSTRACT

The genus *Impatiens* Riv. ex L. (family Balsaminaceae) comprises approximately 1,120 species across the globe, with significant diversity concentrated in the Himalayas and the Western Ghats regions of India. The *Impatiens* are not only valued for their medicinal properties and aesthetic appeal but also for their important ecological roles. However, several *Impatiens* species in India are facing threats due to their habitat specificity and other anthropogenic pressures. Several species of *Impatiens* have been already listed under IUCN threat categories. Thus, recent research efforts have focused on the *Impatiens* taxa. The central Western Ghats region in Karnataka state is a critical habitat for many endemic *Impatiens* species. This study investigated the evolutionary relationships among the *Impatiens* taxa using the nuclear Internal Transcribed Spacer (ITS) region. Phylogenetic analysis performed following the UPGMA method across three hierarchical levels: global (89 taxa), Western Ghats region (53 taxa) and Karnataka state (21 taxa). The results support the hypothesis that south India and Sri Lanka constitute primary centers of origin for the genus *Impatiens*. Within the Western Ghats, multiple speciation events appear to have been driven by geographical isolation and varied microclimatic conditions, particularly in mid-elevation and high-altitude zones. Specifically, within Karnataka, the analysis reveals that older, early-diverged species tend to have wider distributions, while more recently evolved taxa exhibit restricted ranges. The restricted distribution of recently evolved species in Karnataka emphasizes the urgent need for targeted conservation efforts to protect these vulnerable taxa and preserve regional biodiversity.

Keywords : *Impatiens*, Phylogenetic, Western ghats, Evolutionary relationship conservation

INDIA ranks as one of the global megadiverse countries among the world's most biologically rich nations recognized. It is home to four biodiversity hotspots and three major centers of plant endemism, owing to its wide-range of geographic and climatic variation (Plant Discoveries, 2023). Among these, the Western Ghats stands out as a particularly important region, renowned for its exceptional plant diversity and endemism (www.conservation.org; Venkatesh *et al.,* 2019). This mountain range harbors over 7,400 plant species, nearly 4,000 of which are flowering plants and approximately 1,500 are endemic to the region (Nayar *et al.,* 2014 and Venkataraman *et al.,* 2018). Several genera show high levels of endemism to the Western Ghats, India. Among them, the genus *Impatiens* L. (Balsaminaceae) is important one that shows high levels of endemism to this region. Thus, making it a genus of both ecological and conservation significance (Shajitha *et al.,* 2016).

The genus *Impatiens* includes over 1,120 species distributed mainly in tropical and subtropical regions

of the world (Janssens *et al.,* 2006). Belonging to the family Balsaminaceae, this genus is accompanied by just one other genus, *Hydrocera Blume ex Wight & Arn.*, which is monotypic, represented by *Hydrocera triflora* (L.) Wight & Arn. - a semi-aquatic species native to the Indo-Malayan region. *Hydrocera* differs from *Impatiens* in having five free petals and a fleshy, berry-like fruit. However, the genus *Impatiens* exhibits zygomorphic flowers with fused lateral petals and a characteristic five-valved capsule. One unique morphological feature of *Impatiens* is the floral spur- an elongated sepal extension that stores nectar and facilitates pollination (Bhaskar, 2012).

Beyond their botanical interest, *Impatiens* species are widely appreciated for their ornamental and medicinal value. In traditional Indian medicine systems like Ayurveda, Siddha and Unani, various *Impatiens* species have been used to treat ailments such as jaundice, corns and even snakebites (Singh *et al.*, 2015). Pharmacologically, the genus has shown a wide range of activities, including antibacterial, antifungal, antioxidant and anti-inflammatory effects.

Globally, *Impatiens* exhibits five major centers of diversity: Southeast Asia, Southern India and Sri Lanka, Tropical Africa, Madagascar and the Eastern Himalayas. These regions exhibit particularly high endemism-over 90 per cent of *Impatiens* species in Southern India and nearly all in Madagascar, are endemic (Shajitha *et al.,* 2016). In India, *Impatiens* species are mainly concentrated in two regions: the Himalayas and the Western Ghats. The species from these regions can generally be distinguished by fruit morphology-Western Ghats species typically have short, swollen pods, whereas Himalayan species possess long, slender ones (Dessai, 2009). The Western Ghats alone host over 170 taxa of *Impatiens*, with a majority being endemic to the region (Bhaskar, 2012). In Karnataka, which covers the central portion of the Western Ghats, around 70 *Impatiens* taxa have been documented, most of which are endemic (Bhaskar, 2012 and Plant Discoveries, 2023).

Despite the genus's ecological significance and medicinal importance, there has been limited research on the taxonomy, genetic diversity and phylogenetic relationships of Indian *Impatiens*. While studies in Europe have used molecular markers such as RAPD and SSR to examine genetic diversity (Tamboli *et al.,* 2018; Kupcinskiene *et al.,* 2015 and Ramoniene *et al.*, 2016), such approaches remain underutilized in Indian research. SSR markers, in particular, are valuable for exploring genomic variation due to their ability to target microsatellite regions (Zietkiewicz *et al.,* 1994). Molecular phylogenetic studies using nuclear ITS and chloroplast markers have suggested that *Impatiens* likely originated in Southeast Asia and subsequently dispersed to other continents via the Himalayas (Bhaskar, 2012).

Given the high level of endemism and ecological vulnerability of *Impatiens* in the Western Ghats, there is a pressing need for in-depth molecular studies to better understand the phylogenetic relationships among these species. This study aims to explore the evolutionary relationships of endemic *Impatiens* taxa in Karnataka, located in the central Western Ghats, using nuclear ITS sequence data.

Material and Methods

In Karnataka, there are 46 species of *Impatiens* reported; of these 19 species ITS sequence analysis has been already done. The remaining 27 species sequence analysis needs to be done. In this study, sequence analysis of 8 remaining *Impatiens* species was attempted (Table 1). However, out of 8 species collected, only 2 species yielded good PCR amplification. Further, the sequence data of previously published species was also used for the analysis (89 species). To construct phylogenetic tree, totally 89 species data for global analysis, 53 species data for Western Ghats analysis and 21 species data for Karnataka state analysis have been used.

Isolation of genomic DNA from leaf tissue samples : Genomic DNA of the species was isolated following the CTAB method (Allen *et al.,* 2006). The CTAB extraction buffer was prepared by combining specific reagents to a final volume of 100 ml. The specific reagents used includes, the buffer contains 2 per cent

Table 1
List of species and location from the Western Ghats, India

Species Names	Collection site	Geo-coordinates
Impatiens incospinca var. *nematostachys*	Mullayanagiri, Chikkamagaluru	13.387335, 75.706872
Impatiens barberi	Devaramane, Chikkmagaluru	13.061586, 75.538752
Impatiens mullainngiriensis	Mullayanagiri, Chikkmagaluru	13.390449, 75.721069
Impatiens pendula	Mullayanagiri, Chikkmagaluru	13.390446, 75.721069
Impatiens fasciculata	Mullayanagiri, Chikkmagaluru	13.390446, 75.721069
Impatiens mysonensis	Botanical Garden, GKVK	13.07766, 77.574011
Impatiens balsamina var. *parsunathica*	Mullayanagiri, Chikkmagaluru	13.390446, 75.721069
Impatiens scabriscula var. *rosea*	Mullayanagiri, Chikkmagaluru	13.387335, 75.706863

(w/v) Cetyl Trimethyl Ammonium Bromide (CTAB), 100 mM Tris-HCl (pH 8.0) from a 1 M stock solution, 20 mM EDTA (pH 8.0) from a 0.5 M stock and 1.4 M NaCl derived from a 5 M solution. Additionally, 0.2 per cent β-mercaptoethanol is added freshly before use to reduce oxidative damage and 2 per cent polyvinylpyrrolidone (PVP) is included to bind and remove polyphenolic compounds. Alongside this, a 3 M potassium acetate solution was prepared and adjusted to pH 5.2, which was used during the DNA purification process to aid in the removal of proteins and other impurities.

Fresh and healthy leaves collected in the field were wrapped in aluminium foil and after labelling the samples were transported to the laboratory in liquid nitrogen cylinder. In the lab, samples were thoroughly cleaned with distilled water and surface-sterilized using 70 per cent ethanol. Then leaves (200 mg) were crushed into a fine powder using liquid nitrogen in a pestle and mortar. To the powdered tissue, 2 ml of freshly prepared, pre-warmed CTAB extraction buffer was added and the mixture was transferred into a 2 ml Eppen dorf tube. The samples were incubated at 65 °C for 60 minutes in a water bath, with gentle mixing every 15 minutes to ensure uniform extraction. After incubation, the tubes were allowed to cool at room temperature for 10 minutes and then centrifuged at 10,000 rpm for 10 minutes at 4 °C. The supernatant was carefully transferred to a new 1.5 ml Eppendorf tube and an equal volume of phenol: chloroform : isoamyl alcohol (25:24:1 V/V) was added. The mixture was gently inverted 5 to 10 times and centrifuged again at 10,000 rpm for 10 minutes at 4°C. The upper aqueous phase was transferred to a fresh tube and an equal volume of chloroform: isoamyl alcohol (24:1 V/V) was added (repeated twice). To this, 0.6 ml of chilled isopropanol, 0.1 ml of 3 M potassium acetate and 0.1 ml of absolute ethanol were added. The contents were mixed gently by inversion and incubated overnight at -20 °C to allow DNA precipitation. The following day, the samples were centrifuged at 14,000 rpm for 10 minutes at 4 °C, resulting in the formation of a DNA pellet. The supernatant was discarded and the pellet was washed with 600 µl of 70 per cent ethanol, followed by centrifugation at 10,000 rpm for 10 minutes at 4 °C. The DNA pellet was air-dried for 1 to 2 hours to remove any residual ethanol. Finally, the dried DNA pellet was re-suspended in 30-50 µl of sterile water, depending on the pellet size. To remove RNA contamination, 3 µl of RNase A solution was added and the mixture was incubated at 37 °C for 30 minutes. The purified DNA was then stored at -20 °C for future use.

The quality of DNA was assessed by running genomic DNA with 6X loading dye on 0.8 per cent agarose gel electrophoresis in 1X TAE buffer. It was stained using 5µl ethidium bromide (10mg/ml) per 100ml and after migration with 80 V current,

bands were visualized in a UV trans-illuminator. DNA was checked for shearing and contamination of RNA and protein. Finally, the gel image was captured and stored using a gel documentation unit, Alpha Innotech and Fluor Chem® FC2 Imaging System (Department of Biotechnology UAS, GKVK). The DNA concentration and purity were quantified based on A_{260}/A_{280} ratio values obtained by Nanodrop spectrophotometer (Eppendorf). Once the quality and quantity were checked the DNA was diluted to a concentration of 50ng/µl and further used for PCR reactions.

PCR analysis : The primers used for the amplification of the ITS gene were ITS 4- 5'-GGAAGTAGA AGTCGTAACAAGG-3' and ITS 5- 5'-TCCTCCG CTTATTGATATGC-3' (White *et al.,* 1990). The temperature profile consisted of 2 min initial denaturation at 95°C followed by 35 cycles of 30 s denaturation at 95°C, 30 s primer annealing at 50.9°C, 1 min extension at 72°C and a final extension of 8 min at 72°C. The PCR product amplification was performed in a 0.2 mL PCR tube containing a 20µL reaction mixture with components mentioned in Table 2. Polymerase Chain Reaction (PCR) was carried out in the Eppendorf Mastercycler Nexus Gradient instrument. The PCR conditions for amplification of primers are given in Table 3. The annealing temperature was standardized for selected primers using PCR conditions.

Table 2

Components of the reaction mixture for PCR amplification

Components	Concentration	Volume in 20µl stock
EmeraldAmp GT PCR Master Mix	2X(Premix)	10
ITS-5 Forward primer	10 pMol	1
ITS-4 Reverse primer	10 pMol	1
Genomic DNA	50 ng	2
Sterile water	-	6

Table 3

PCR reaction condition for amplification of genomic DNA using ITS primers

Steps	Temperature (°C)	Duration	Cycles
Initial denaturation	95.00	2 min	35
Denaturation	95.00	30 sec	
Annealing	50.9*	30 sec	
Extension	72.00	1 min	
Final extension	72.00	8 min	
Hold	4.00	5 min	

** Annealing temperature (Shajitha et al., 2016)*

Agarose gel electrophoresis and Sequencing : Amplified products (20µl) were loaded on 3.5 per cent agarose gel in 1X TBA buffer as electrolyte. 100 bp step-up ladder was used as a molecular weight marker to estimate amplicon size. Gels were viewed under the gel documentation system, Alpha Innotech and FluorChem® FC2 Imaging System. The PCR amplified product was outsourced to Medauxin company for gene sequencing. The sequence reads were received in fasta format and were used for sequence analysis by carrying out BLAST (Altschul *et al*., 1997) analysis against the nr database at NCBI.

Phylogenetic Analysis : For the phylogenetic analysis, *Hydrocera trifolia* was used as outgroup (following Yu *et al.,* 2016). The sequences obtained from the Medauxin Pvt. Ltd., were first edited using BioEdit softwere and then aligned using Clustal W. Afterwards using the MEGA 11 software analysis was performed. UPGMA (unweighted pair group method with arithmetic mean) algorithm method was adopted. Tree strength was validated by performing bootstrap analysis (1000 replications) (Shajitha *et al.,* 2016). The phylogenetic analysis performed following the UPGMA method across three hierarchical levels: global (89 taxa), Western Ghats region (53 taxa) and Karnataka state (21 taxa, Table 4).

TABLE 4

List of species with DNA sequences accession number obtained from NCBI

Species name	Gene bank Accession Number
Impatiens acaulis	KR011120
Impatiens levingei	KT225490
Impatiens modesta	KT225493
Impatiens pandata	KT225494
Impatiens scapiflora	KJ685214
Impatiens jerdoniae	KT225492
Impatiens violacea	KT225495
Impatiens chinensis	KF804101
Impatiens dalzellii	KT225491
Impatiens debilis	KF804102
Impatiens gardneriana	KF719156
Impatiens herbicola	KF719158
Impatiens ligulata	KF719159
Impatiens lucida	KF719160
Impatiens minor	KF719162
Impatiens oppositifolia	KT254232
Impatiens raziana	KT254235
Impatiens rufescens	KT254236
Impatiens tomentosa	KT254238
Impatiens bababudenensis	KT254226
Impatiens balsamina	KF804100
Impatiens cuspidata	KF719153
Impatiens dasysperma	KJ685213
Impatiens flaccida	KT254228
Impatiens floribunda	KF719155
Impatiens latifolia	KT254231
Impatiens mysorensis	KF719164
Impatiens pulcherrima	KT254234
Impatiens scabriuscula	KF719168
Impatiens walleriana	KF804104
Impatiens henslowiana	KT254230
Impatiens johnii	KF804103
Impatiens neo-munronii	KF719165
Impatiens anaimudica	KT254225
Impatiens cordata	KT254227

Continued....

TABLE 4 Continued....

Species name	Gene bank Accession Number
Impatiens disotis	KF719154
Impatiens goughii	KF719157
Impatiens grandis	KT254229
Impatiens travancorica	KT254239
Impatiens umbellata	KT254240
Impatiens uncinata	KT254241
Impatiens viscida	KF719166
Impatiens viscosa	KF697232
Impatiens maculate	KF719161
Impatiens phoenicea	KT254233
Impatiens tangachee	KT254237
Impatiens wightiana	KF719167
Impatiens aureliana	AY348747
Impatiens bicornuta	AY348754
Impatiens clavigera	HQ718766
Impatiens monticola	AY348810
Impatiens napoensis	AY348811
Impatiens omeiana	KC905505
Impatiens platypetala	AY348819
Impatiens stenosepala	AY348835
Impatiens trichosepala	AY348843
Impatiens xanthine	AY348850
Impatiens bequaerti	AY348753
Impatiens bombycina	AY348755
Impatiens burtonii	AY348757
Impatiens congolensis	AY348766
Impatiens columbaria	AY348764
Impatiens hoehnelii	AY348792
Impatiens keilii	AY348798
Impatiens kilimanjari	AY348800
Impatiens niamniamensis	AY348812
Impatiens stuhlmannii	AY348836
Impatiens auricoma	AY348748
Impatiens baroni	AY348751
Impatiens fuchsioides	AY348785

Continued....

Table 4 Continued....

Species name	Gene bank Accession Number
Impatiens furcata	AY348786
Impatiens gibbosa	AY348787
Impatiens inaperta	AY348797
Impatiens percrenata	AY348817
Impatiens subabortiva	AY348837
Impatiens tuberosa	AY348844
Impatiens vilersi	AY348848
Impatiens repens	KC905524
Impatiens amphorata	AY348740
Impatiens glandulifera	AY348788
Impatiens brachycentra	AY348756
Impatiens parviflora	AY348816
Impatiens nolitangere	AY348813
Impatiens capensis	AY348759
Impatiens leschenaultia	AY348803
H. triflora (out group)	AY348853

Results and Discussion

In India, the genus is represented by 210 species, which are predominantly restricted to either the Himalayan region or the Western Ghats (Jyosna and Janarthanam, 2011). Of these, 170 taxa are reported from the Western Ghats, with 161 taxa being endemic to the region and only nine taxa exhibiting a wider distribution.

Over the years, various hypotheses have been proposed regarding the origin of *Impatiens*. Based on karyological and species diversity data, Jones *et al*. (2021) suggested a Himalayan origin. In contrast, Grey-Wilson (1980) proposed Western Gondwana as the center of origin. An ITS-based phylogenetic study by Yuan *et al.* (2004) indicated Southeast Asia as the most likely origin, a view further supported by subsequent studies (Shajitha *et al.,* 2016 and Tamboli *et al.,* 2018).

The present study aimed to analyze the phylogenetic relationships among *Impatiens* taxa across three levels: (1) a global-level analysis involving 89 taxa, (2) a regional-level analysis focusing on 53 taxa from the Western Ghats and (3) a local-level analysis of 21 endemic taxa from Karnataka, India. *Hydrocera triflora* was used as the outgroup, following the approach of Yu *et al*. (2016). The number of taxa included at each level varied based on data availability and regional representation.

Phylogenetic Analysis of *Impatiens* Taxa Across the World

The global phylogenetic analysis of 89 *Impatiens* taxa revealed the presence of nine well-supported clades, each broadly corresponding to distinct geographic regions, as indicated by robust bootstrap values (Fig. 1).

Clade 1, consisting of *I. congolensis* and *I. niamniamensis*, has a bootstrap value of 89 and represents closely related African species.

Clade 2, which includes Malagasy species like *I. fuchsioides* and the African *I. burtonii*, is supported by bootstrap values of 97-99, indicating strong clustering and potential historical dispersal between Africa and Madagascar.

Clade 3, comprising species such as *I. cupidata* and *I. dasysperma* from South India and Sri Lanka, shows a shared lineage with bootstrap support of 80-89, reflecting a geographically confined evolutionary relationship.

In Madagascar, Clade 4, including species like *I. auricoma* and *I. gibbsiana*, is supported by bootstrap values of 81-99, indicating high genetic differentiation and adaptive divergence within the region.

Clade 5, spanning South India, Sri Lanka, East and Southeast Asia, includes species such as *I. aureliana* and *I.scabriuscula*, with a wide range of bootstrap values (62-100) suggesting ancient lineage divergence or limited dispersal events.

Clade 6, confined to South Indian and Sri Lankan species like *I. wightiana* and *I. phoenicea*, has strong

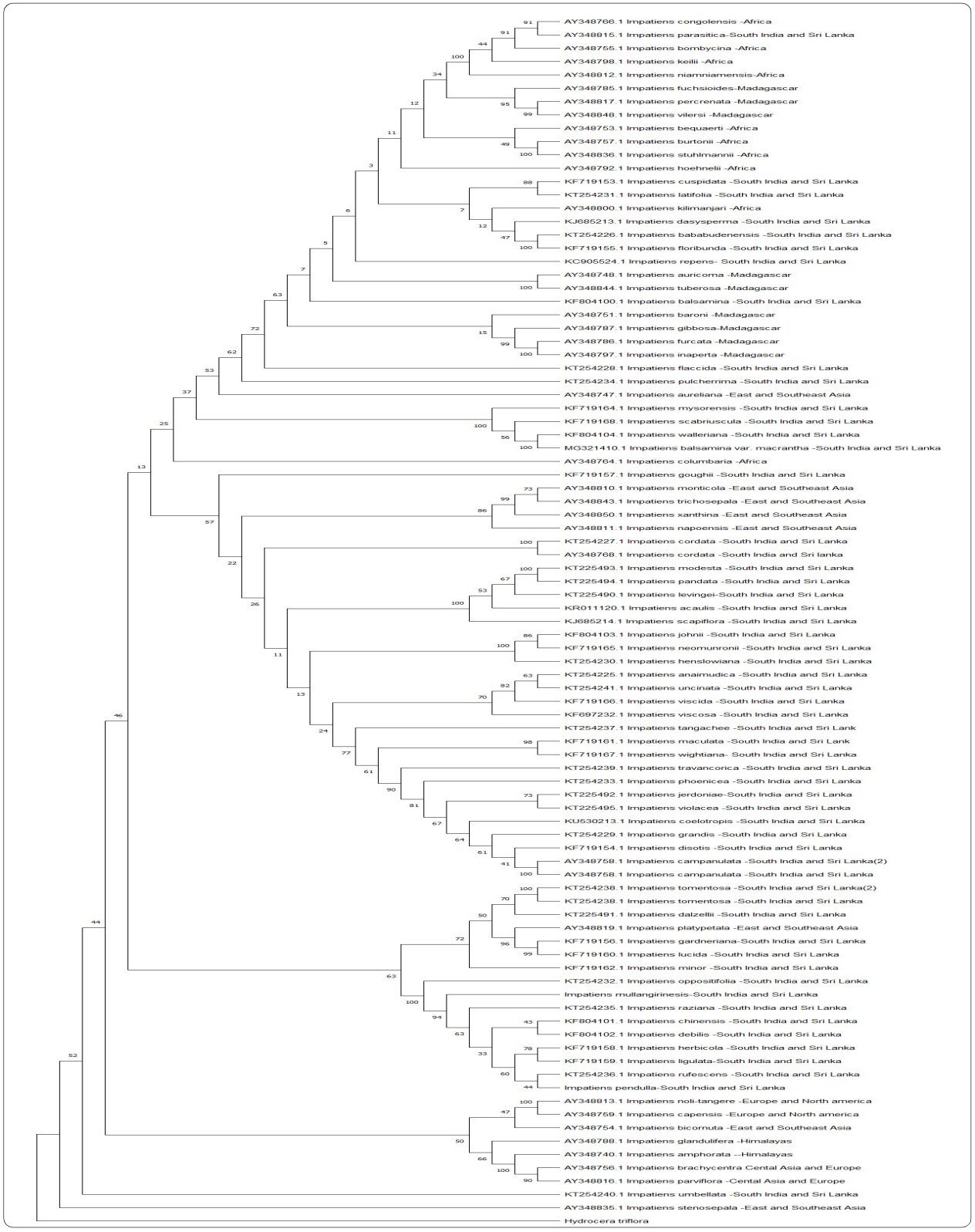

Fig. 1 : Consensus phylogenetic tree of world from the UPGMA analysis based on nuclear ITS gene. The numbers by the nodes indicate UPGMA bootstrap support values greater than 50%

support (85-100), underscoring significant speciation in this biodiversity hotspot.

Clade 7, comprising *I. chinensis* and *I. debilis*, reflects connections between South Asian and East Asian species, likely due to historical migration, with bootstrap values of 92-100.

Clade 8, which includes species from Central Asia, Europe and South India, such as *I. rufescens* and *I. campanulata*, shows mixed clustering with bootstrap support of 50-98, suggesting shared ancestral lineages and subsequent regional adaptation.

Clade 9, with species like *I. bicornuta* and *I. stenosepala* from East and Southeast Asia, has bootstrap values ranging from 53 to 100, emphasizing strong regional clustering and diversification.

The outgroup *Hydrocera triflora* is clearly resolved as a distinct lineage, confirming its divergence from the *Impatiens* genus and validating its status as a separate taxonomic entity.

Overall, the phylogenetic analysis indicates that South India and Sri Lanka are likely primary centers of evolution and diversification for the genus *Impatiens*. This region harbors the highest concentration of closely related species, as reflected by their presence across multiple well-supported clades. These patterns point to extensive speciation events, likely driven by unique ecological conditions, prolonged geographic isolation and long-term evolutionary processes. Similar studies in wild *Cymbopogon martinii* showed influence of environmental factors for particular adaptation (Hemalatha *et al*. 2022).

Additionally, Madagascar emerges as another significant evolutionary hotspot, with distinct clades exhibiting high genetic differentiation and signs of adaptive radiation. The observed phylogenetic links between South Asia, East Asia and Southeast Asia suggest historical dispersal events or shared ancestral lineages. However, the dominance of South Indian and Sri Lankan species within the phylogeny underscores the critical role of this region in shaping the evolutionary trajectory of *Impatiens*.

These findings strongly support the hypothesis that South India and Sri Lanka represent the true centre of origin for the *Impatiens* genus, aligning with the conclusions of Yuan *et al*. (2004), Shajitha *et al*. (2016) and Tamboli *et al*. (2018).

Phylogenetic Analysis of *Impatiens* Taxa of Western Ghats, India

Out of the 170 *Impatiens* taxa reported from the Western Ghats, India, molecular data was available for 53 taxa. Phylogenetic analysis was carried out using the UPGMA method (Fig. 2), which revealed the existence of six distinct clades, each well-supported by bootstrap values.

The Clade 1 (bootstrap: 66) comprises *I. disotis, I. grandis, I. campanulata, I. coelotropis, I. jerdoniae, I. violacea, I. phoenicea, I. travancorica, I. maculata, I. wightiana, I. tangachee, I. henslowiana, I. johnsii* and *I. neomunronii.* Notably, there is strong internal support for *I. johnsii and I. neomunronii* (bootstrap: 100), indicating closely related species with overlapping distributions. Species found in the Kerala's montane environments, including Anamudi Peak and the Travancore High Range. In Tamil Nadu's biodiversity-rich high-altitude regions, such as Kodaikanal and Nilgiri Hills and in the Karnataka's isolated hill ranges, including Kodagu, B.R. Hills and Jog Falls. These species are associated with high altitude.

Clade 2 (bootstrap: 72) includes *I. scapiflora, I. acaulis, I. levingei, I. modesta* and *I. pandata.* These species are found in the mid-elevation montane forests of Karnataka and Tamil Nadu.

Clade 3 (bootstrap: 57) encompasses *I. cordata, I. goughii, I. viscosa, I. viscida, I. anamudica* and *I. uncinata*. These species are found in the mid-to-lower altitudes of the Western Ghats, the regions of Karnataka and Kerala.

Clade 4 (bootstrap: 99) includes *I. gardneriana, I. lucida, I. minor, I. dalzellii, and I. tormentosa,* with robust support for the subgroup of *I. gardneriana* and *I. lucida* (bootstrap: 100). These species are found in mixed regions such as in wet forest habitats,

Palakkad and Wayanad regions of Kerala and Karnataka.

Clade 5 (bootstrap: 81) comprises *I. walleriana, I. balsamina var. macrantha, I. scabriuscula, I. mysorensis, I. pulcherrima, I. flaccida, I. balsamina, I. parasitica, I. cuspidata, I. latifolia, I. dasysperma* and *I. bababudenensis*. This clade shows strong internal support, particularly for *I. dasysperma* and *I. bababudenensis (bootstrap: 76)* and *I. walleriana* with *I. balsamina var. macrantha* (bootstrap: 99). The species in this group are found in the mid elevation of Maharashtra and high altitude peak lands of 'Karnataka and Tamil Nadu'.

Finally, Clade 6 (bootstrap: 100) consists of *I. oppositifolia, I. mullangiriensis, I. chinensis, I. raziana, I. debilis, I. pendula, I. rufescens, I. herbicola* and *I. ligulata*. These species are found in the Tamil Nadu's Anamalai Hills. The one species*, I. umbellata* was found independent and not grouped under any of the clad.

The species were clustered based on the geography and the altitude. The speciation event has occurred many times in the Western Ghats. It appears that speciation is based on the habitat, as and when the ecological and environmental conditions varied, that has led to species evolution. In conclusion, geographical isolation and diverse microclimates within the Western Ghats, particularly in high-altitude zones and mid-elevation plateaus, have played a crucial role in driving the speciation and endemism of *Impatiens*. With regards evolution of species from wide spread to restricted ones and vice versa. In Western Ghats both the events are possible, in the present study clad 2, clad 4 and clad 6 is observed that species have evolved from widespread to restricted habitat. In clad two *I. scapiflora* and *I. acaulis,* are widely distributed in the Western Ghats and the newly evolved species such as *I. modesta* and *I. pandata* have more restricted distribution.

The phylogenetic evidence highlights the role of geographical isolation and habitat heterogeneity in shaping the evolutionary history of *Impatiens* in the Western Ghats. The region serves as a dynamic center for both diversification and speciation, driven by its complex topography and environmental gradients. The observed evolutionary transitions-from broadly distributed species to narrowly endemic ones-highlight the ongoing and adaptive nature of *Impatiens* evolution in this globally significant biodiversity hotspot.

Similarly, in the clad 1, clad 3 and clad 5 the speciation has occurred from restricted to widespread distribution within the Western Ghats, India. The species namely *I. johnsii* and *I. neomunronii* have more restricted distribution and from this species such as *I. disotis* and *I. grandis* have evolved that have more wider distribution within the Western Ghats. Similar result align with Tambat (2014) with *Myrictica* swamps where speciation from restricted distribution to widespread distribution.

Phylogenetic Analysis of *Impatiens* taxa of Karnataka

Out of the 46 known *Impatiens* taxa reported from Karnataka, molecular data had previously been published for 19 species. In this study, molecular data were generated for two additional species, increasing the total to 21 taxa. Using ITS sequence data, a phylogenetic tree was constructed to explore the evolutionary relationships among these taxa (Fig. 3). The phylogenetic analysis revealed five distinct clades, each representing varying degrees of evolutionary divergence:

Clade 1, the most basal grouping, comprises three subclades. Subclade 1.1 includes *I. jerdoniae* and *I. viscida*, which share a moderately close evolutionary relationship with a bootstrap value of 70. Subclade 1.2 features *I. goughii* and *I. acaulis*, exhibiting a strong evolutionary link with high confidence supported by a bootstrap value of 85. Subclade 1.3 groups *I. scapiflora* and *I. henslowiana*, which display a strong evolutionary connection, with a bootstrap value of 80. Clade 2 contains species, including *I. gardneriana* and *I. minor*, which form a pair with a moderately strong evolutionary link indicated by a bootstrap value of 70.

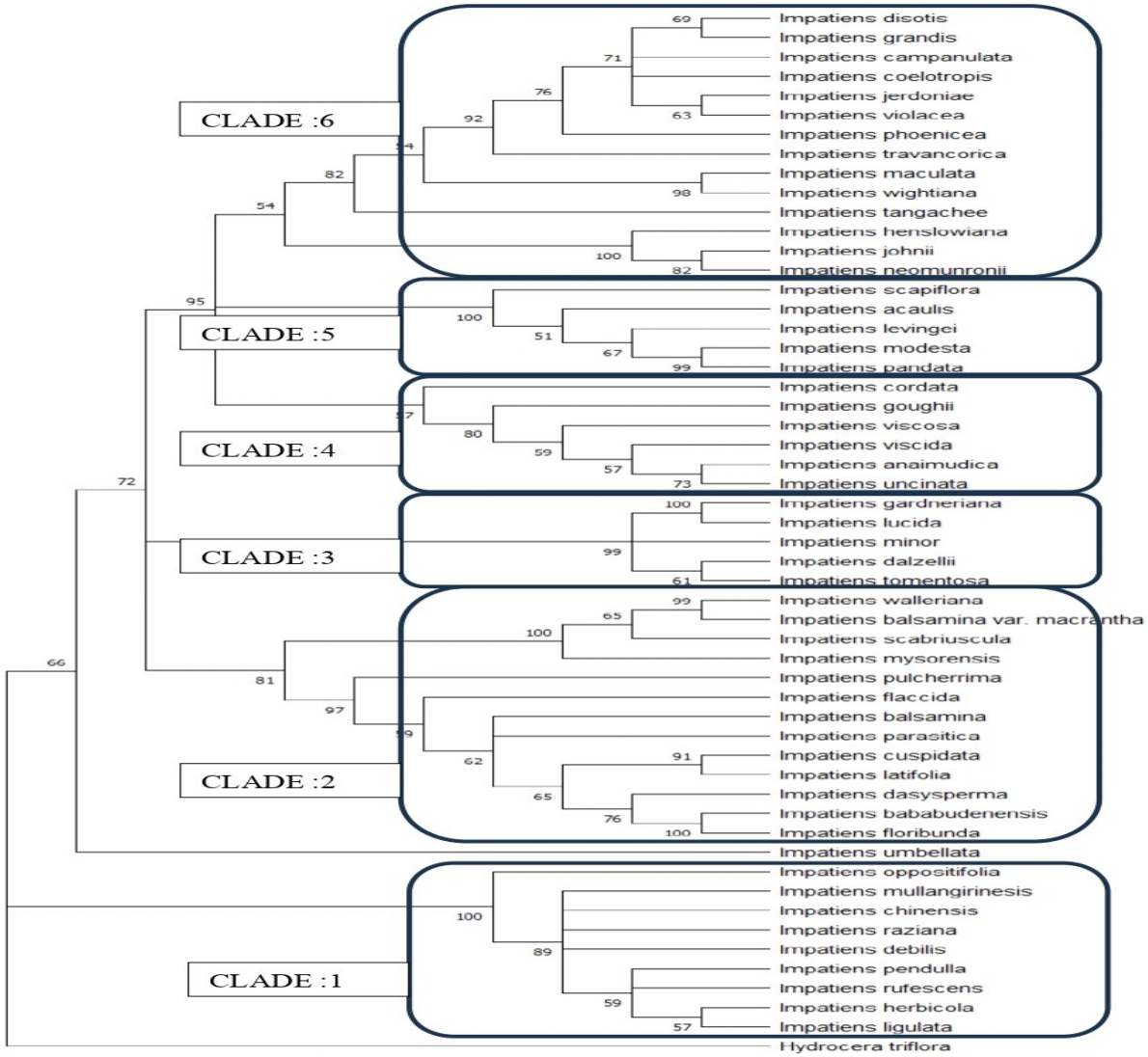

Fig. 2 : Consensus phylogenetic tree of Western Ghats from the UPGMA analysis based on nuclear ITS gene. The numbers by the nodes indicate UPGMA bootstrap support values greater than 50%

Clade 3 represents species with very strong evolutionary relationships. Subclade 3.1 includes *I. pulcherrima* and *I. balsamina*, which show a very close evolutionary relationship strongly supported by a bootstrap value of 98. Subclade 3.2 features *I. bababudenensis* and *I. dasysperma*, forming a strongly supported subgroup with a bootstrap value of 82.

Clade 4, divided into three subclades. Subclade 4.1 includes *I. lawii* and *I. oppositifolia*, which show a moderate evolutionary link with a bootstrap value of 63. Subclade 4.2 groups *I. tomentosa* and *I. chinensis*, supported by a bootstrap value of 67. Subclade 4.3 includes *I. raziana* and *I. pendula*, exhibiting a weak to moderate evolutionary relationship with a bootstrap value of 57.

Finally, Clade 5 groups species with close evolutionary relationships. This clade includes *I. balsamina var. macrantha*, *I. scabriuscula* and *I. mysorensis*, which show a very close evolutionary relationship, strongly supported by a bootstrap value of 100.

Tamboli *et al.,* (2018) constructed a phylogenetic tree for 10 *Impatiens* species using UPGMA dendrogram

analysis based on ISSR marker data, revealing four distinct groups. Group I included *I. lawii*, *I. tomentosa*, and *I. dalzellii*, with *I. tomentosa* and *I. dalzellii* belonging to the Annuae section, while *I. lawii* was placed in the Oppositifolia section. Group II consisted of *I. rosea*, *I. balsamina* var. *macrantha* and *I. acaulis*, where *I. rosea* and *I. balsamina* var. *macrantha* were part of the Microcepalae section and *I. scapiflora* belonged to the Scapiflora family. Group III contained *I. pulcherrima*, *I. minor* and *I. oppositifolia*, with *I. minor* and *I. oppositifolia* in the Annuae section and *I. pulcherrima* in the same section. *Impatiens walleriana* formed a separate group, Group IV.

Our phylogenetic analysis identified five distinct groups, each comprising species from seven sections. Group 1 includes eight species: *I. jerdoniae*, *I. viscida* and *I. goughii*. While *I. viscida* and *I. goughii* belong to the section *Sub-umbellatae*, *I. jerdoniae* is classified under the section *Epiphyticeae*. Group 2 consists of three species: *I. acaulis*, *I. scapiflora* and *I. henslowiana*. Among these, *I. acaulis* and *I. scapiflora* belong to the section *Scapiflora*, whereas *I. henslowiana* is part of the section *Tomentosa*. Group 3 includes *I. minor* and *I. gardemariae*, both of which belong to the section *Annuae*. Group 4 comprises *I. pulcherrima*, *I. balsamina*, *I. bababudenensis* and *I. dasysperma*, all of which are classified under the section *Microcepalae*. and also consists of *I. lawii*, *I. oppositifolia*, *I. tomentosa*, *I. chinensis*, *I. mullaringensis*, *I. raziana*, *I. pendula* and *I. herbicola*. Among these, *I. lawii*, *I. oppositifolia*, *I. tomentosa*, *I. chinensis*, *I. mullaringensis* and *I. raziana* belong to the section *Annuae*, while *I. pendula* and *I. herbicola* are classified under the section *Microcepalae*.

The phylogenetic analysis of *Impatiens* taxa suggests that South India and Sri Lanka are the true origin of the genus. In the Western Ghats, speciation has occurred multiple times, influenced by geographical isolation and diverse microclimates. High-altitude zones and mid-elevation plateaus have played a crucial role in species evolution and endemism. Early-evolved taxa remain widespread, while recently evolved taxa exhibit habitat specificity, leading to restricted distributions. This pattern indicates an ongoing evolutionary process driven by ecological and environmental variations. Overall, the study highlights the dynamic nature of *Impatiens* evolution in the Western Ghats.

Finally, Group 5 includes *I. scabriscuta*, *I. mysorensis* and *I. balsamina* var. *macrantha*, all of which belong to the section *Microcepalae*. Overall, our findings demonstrate a partial alignment with Tamboli's results and Hooker's classification system, underscoring the complexity of phylogenetic relationships within these taxa.

The phylogenetic analysis of Karnataka taxa suggest that taxa located near the outgroup evolved earlier. These early-evolved taxa are distributed throughout the Western Ghats. However, due to factors such as climate change or anthropogenic interventions, the continuous distribution of taxa in the Western Ghats has been disrupted. This disruption could have led to speciation, perhaps due to local adaptations corresponding to their respective habitats. Consequently, the newly evolved taxa have developed specific habitat preferences, while the early-evolved taxa remain widespread across the Western Ghats. Secondly, most of them are herbaceous and season bound this could make them more endemic ones. This habitat specificity and preference makes *Impatiens* taxa endemic to the region.

The study indicate that within clade 1, *I. henslowia* represents an early evolved species that is distributed across multiple locations within the Western Ghats. In contrast, *I. jerdoniae*, is restricted to a specific region within the Western Ghats. This means widespread species evolved from the restricted distribution species. Our pattern of discovery analysis also suggests that earlier evolved taxa tend to be widespread throughout the Western Ghats, whereas more recently discovered taxa are often confined to particular regions. These results align, with the findings of Arvind *et al*. (2007), which suggest that widespread taxa are discovered

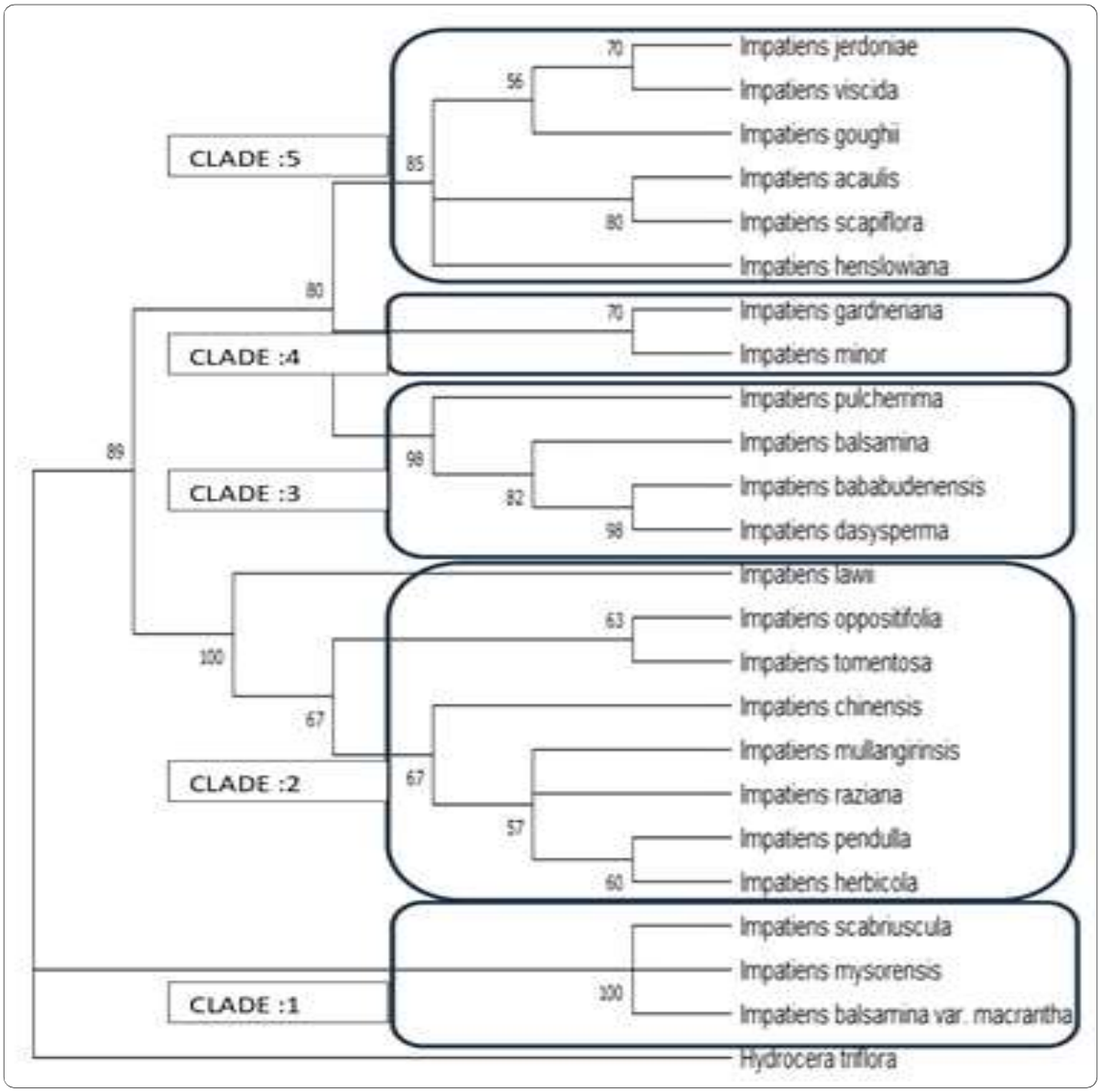

Fig. 3 : Consensus phylogenetic tree of Karnataka from the UPGMA analysis based on nuclear ITS gene. The numbers by the nodes indicate UPGMA bootstrap support values greater than 50%

earlier, while endemic taxa have been identified in more recent years.

The Western Ghats' steep elevational gradients, varying rainfall regimes and dissected topography (massifs, gaps such as the Palghat Gap) create many micro-climatic niches and geographic barriers. For example: the Palghat Gap is cited as a barrier in the WG inhibiting gene flow. These conditions lead to repeated episodes of population isolation (geographic) and adaptation to local microclimates. Over evolutionary time this drives lineage divergence (speciation) and high endemicity. Multiple taxa (frogs, birds, plants) show endemic radiations in the WG tied to such environmental and topographic heterogeneity (Vijayakumar, *et al.,* 2016; Joshi *et al.,* 2020 and Aravind *et al.,* 2023).

This study reaffirms that South India and Sri Lanka are likely the true centers of origin and diversification

for the genus *Impatiens*. Phylogenetic analyses at global, regional and local scales reveal distinct, well-supported clades shaped by geography, altitude and ecological conditions. The Western Ghats, in particular, show repeated speciation events driven by microclimatic variation and geographical isolation. Early-evolved taxa tend to be widespread, while recently evolved species exhibit habitat specificity and restricted distributions-highlighting ongoing evolutionary processes. The herbaceous and seasonal nature of *Impatiens* further contributes to their endemism.

The Western Ghats represent not only a biodiversity hotspot but also a crucial center of evolutionary activity for *Impatiens*. Conservation efforts should prioritize high-altitude and mid-elevation habitats, as these areas harbor the highest levels of endemism and active speciation. Additionally, expanding molecular studies to include under represented taxa will enhance our understanding of the genus's lineage relationships and aid in clarifying taxonomic classifications. Overall, this study highlights the significant evolutionary, ecological and conservation value of *Impatiens* in the Western Ghats and surrounding regions.

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Relevance of Root System Architecture for Abiotic Stress Tolerance in Finger Millet [*Eleusine coracana* (L) Gaertn.]

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Finger millet is a staple food and fodder crop predominantly cultivated in rainfed situations, thus affected by the drought stress in the climate change phenomenon and necessitates the identification of drought adaptive genotypes. Of the several contributing parameters to drought adaptation, different root traits in combination called as root system architecture (RSA) could be appropriate. In the present study, 350 finger millet accessions were evaluated for RSA in 37 days old seedlings. The most important component traits that contributed to the genetic diversity in RSA were root dry weight (45.1%) and root length (29.7%) measured manually; and the total root length (8.2 %) measured using a root scanner. There was no correlation between manual and root scanner measurements for all the RSA traits excepting for the root fresh weight with total root length (0.467) and root length density (0.467); however, many root traits of RSA (which are not possible to measure manually) can be measured accurately and easily in less time period, using the root scanner. Under field-imposed drought stress, the contrasting accessions selected for high and low RSA differed significantly only for the leaf temperature and canopy temperature, but not for SCMR, leaf folding, leaf angle, yield and yield attributes under DS conditions. Under the osmotic stress conditions (PEG and NaCl) also, the high and low root types did not differ significantly. However, the high root type accessions (high root dry weight: GE-2275, GE-2572, GE-3516, GE-554; manually measured deep root length: GE-3371, GE-2129, GE-3096, GE-5003, GE-595, GE-5148; and root scanner-measured longer total root length: GE-1417, GE-5134, GE-4172, GE-3096) superior to check varieties GPU-28 and PR-202 under *in vitro* conditions can be used as donors for improvement of RSA and better drought adaptation of finger millet.

Development and Standardization of Synbiotic Fermented Oyster Mushroom Beverage

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This study aimed to develop a synbiotic fermented oyster mushroom (*Hypsizygus ulmarius*) beverage, combining the nutritional benefits of mushrooms with the functional properties of probiotics and prebiotics. In fermentation process probiotics (*Lactobacillus plantarum* and *Lactobacillus delbrueckii*) and prebiotics (Honey and Dates) were used to improve nutrient bioavailability, gut health, immune modulation and antioxidant benefits and different mushroom quantities (10, 20, 30, 40 g) and fermentation durations (1–5 days) were used. The combination of 20 g mushrooms and 4 days fermentation was found to be best with highest acceptability score of 13.15. Further standardization with different concentrations (20, 25 and 30 %) of honey and dates and in combination of both were carried out. The results revealed that 25 per cent honey formulation inoculated with six mL of *Lactobacillus plantarum* produced the most acceptable score (AS). With a balanced pH of 3.6, total soluble solids (TSS) of 10.68 °Brix and titratable acidity (TA) of 1.34 per cent, enhanced sensory qualities of the beverage. By addition of different flavoring agents, use of lemongrass oil (0.1 mL) improved the flavor, increasing AS to 17.53. Microbial population of *Lactobacillus plantarum* maintained a viable population of 31.67×10x CFU/mL under refrigeration up to seven weeks and a significant decline occurred under ambient storage. Biochemical analysis showed minimal reduction in pH and TSS with slight increase in TA over storage, indicating product stability under refrigeration. The final product demonstrated improved nutritional content, including higher levels of vitamins (B_2, B_5, B_9, D), minerals (Fe, Mg, P, K) and bioactive compounds offering significant health benefits such as antioxidant activity and immune modulation. The cost of production was Rs.18.27/150 mL, with Benefit-Cost Ratio of 1.25, indicating strong economic viability. The developed synbiotic fermented mushroom beverage would be a promising potential as a nutritious, health-promoting functional beverage.

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Diversity of Microbes Associated with *Jeevamrutha* for Plant Growth Promotion

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THIS study explored microbial diversity in *jeevamrutha* prepared from various cow breeds and its potential for plant growth promotion. *Jeevamrutha* showed decreasing pH and rising EC while, microbial population particularly fungi and actinomycetes increased over time, during incubation period. *Jeevamrutha* prepared from Hallikar, Deoni, Gir and Ongole had the highest nitrogen (1.91%), phosphorus (0.24%), potassium and copper (0.31% and 1.70ppm) and organic carbon and zinc (20.77% and 5.10 ppm), respectively. A novel *jeevamrutha*-based medium supported better bacterial and actinomycetes isolation. Metagenomic analysis revealed that, phylum Firmicutes were dominant across *jeevamrutha* samples. *Clostridium butyricum* was dominant in *jeevamrutha* prepared from Deoni (12.35%) and Ongole (5.64%) while, *Lactobacillus mucosae* (5.24%) was abundant in Gir. Metabolomics showed that, Deoni and Gir *jeevamrutha* were abundant in secondary metabolites like butanoic acid (37.30 and 68.32%, respectively) while, Ongole *jeevamrutha* was abundant in tetradecanoic acid (34.48%). Among thirty bacterial isolates, highest phosphate solubilization, exopolysaccharide, ammonia, salicylic acid, FPase, endoglucanase, β-glucosidase and xylanase production were recorded in D-12 (12.15µg/mL), H-8 (1.50µg/mL), D-12 (10.65 µg/mL), H-8 (0.013µg/mL), G-10 (0.76 IU/mL), D-15 (0.59 IU/mL), H-13 (0.37 IU/mL) and D-16 (0.18 IU/mL), respectively. Efficient isolates were identified as *Paenibacillus* sp. strain H11_UASB, *Paenibacillus typhae* strain H13_UASB and *Bacillus* sp. strain D16_UASB. The consortium of these three was evaluated under different farming practices in ragi. RDF recorded highest yield (T_2: 2940.00 kg/ha) and microbial population. Organic farming (T_3) recorded highest dehydrogenase (91.41 µg TPF g^{-1} soil h^{-1}), acid and alkaline phosphatase (31.67 and 12.37 µg PNP g^{-1} soil h^{-1}), urease (37.01 µg NH_4^+-N g^{-1} soil h^{-1}) enzyme activity, organic carbon (0.36%) while, RDF (T_2) showed highest nutrient availability (N: 223.31 kg/ha, P_2O_5: 42.32 kg/ha, K_2O: 185.50 kg/ha) and plant nutrient uptake (N: 84.73 kg/ha, P_2O_5: 36.79 kg/ha, K_2O: 56.96 kg/ha) at harvest.

Studies on Effect of Endophytes on Plant Growth Promotion and Biocontrol Activities in Mung Bean (*Vigna radiata*)

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THE current study was designed and carried out to harness the plant growth promotion and biocontrol activities of endophytes against the fungal pathogen *Macrophomina phaseolina* which causes dry root rot disease in green gram. A total of 21 bacterial and 15 fungal endophytes were isolated from various plant parts and rhizosphere soil of green gram collected from four different locations. All the isolated endophytes were morphologically characterized and were primarily screened against *Macrophomina phaseolina* by dual culture method to know the inhibition percentage *in vitro*. Further, 9 selected (3 fungal and 6 bacterial) endophytes were subjected to secondary screening for plant growth promotion traits. Two isolates RE- 2 (Fungal) and STE-4 (Bacterial) exhibited superior results for all the tests and were identified as *Trichoderma viride* RE-2 and *Pseudomonas furukawaii* STE-4, respectively upon molecular sequencing. Further, these two efficient isolates were treated to green gram plants in pots under greenhouse conditions, the disease incidence significantly decreased in the treatment T_{12} (Seed treatment with *Trichoderma viride* and soil drenching with *Pseudomonas furukawaii*) (8.04%) compared to pathogen treated (89.9 %). In addition to biocontrol activity, T_{12} recorded significantly higher plant growth and yield attributes *viz.,* plant height (26.24cm), number of branches per plant (5.32 branches) and pod yield (11.74 g/plant) and also recorded highest plant nutrient uptake at harvest (56.20 mg/plant) of N, (25.93 mg/plant) of P, (32.35 mg/plant) of K. Thus, these endophytes offer a multifaceted application as they inhibit the disease and enhance the plant growth and development.

Plant Growth Promotion and Biocontrol Activity of Endophytes on *Macrophomina phaseolina* in Cowpea (*Vigna unguiculata*)

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THE endophytes associated with cowpea were isolated, characterized and screened for plant growth promotional and biocontrol activity. Out of 30 bacterial and 9 fungal endophytes isolated, based on their ability to produce phytohormones, performance in various biochemical tests and biocontrol activity against *Macrophomina phaseolina,* one bacteria *Bacillus subtilis* and one fungal isolate *Trichoderma harzianum* has been selected. *In vitro* screening of isolates was carried out by dual culture method against the cowpea dry root rot pathogen (*Macrophomina phaseolina*). The per cent growth inhibition by *B. subtilis* (61.40%) and *T. harzianum* (61.04%) was recorded. A pot experiment was conducted under greenhouse conditions at the Department of Agricultural Microbiology, GKVK, UAS, Bangalore with 14 treatments and 3 replications consisting of seed treatment and soil drenching along with RDF of 20:40:20 of NPK. The treatments T_7 and T_8 recorded significantly higher growth and yield attributes *viz*., plant height (141.54 and 132.54cm), number of branches per plant (25.24 and 24.97 branches) and pod yield (109.07 and 97.33 g/plant), respectively and also recorded higher plant nutrient uptake at harvest (55.87 mg/plant) of N, (26.85 mg/plant) of P and (34.58 mg/plant) of K and also treatment T_7 has recorded highest number of root nodules (30.65) followed by T_8 (28.98). The application of these isolates improved crop growth and yield by controlling the dry root rot disease.

Molecular Genetic Improvement of Productivity Traits in Papaya cv. Arka Prabhath by Tilling

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ARKA PRABHATH is an advanced gynodioecious cultivar of papaya developed at ICAR-IIHR, Bengaluru. Mutational influence on fruit quality traits was studied by treating the control seeds with different doses (0 Gy, 50 Gy, 100 Gy, 150 Gy, 250 Gy and 500 Gy) of gamma irradiation, using Co-60 as a radiation source. With a germination rate of 50.63 per cent, the LD50 for gamma radiation was 50 Gy. T_2 (50 Gy) of the M_1 generation had the smallest first-flowering height (54 cm), while T_4 (150 Gy) had the earliest flowering (average: 88 days). Dwarf stature plants were observed comparatively more in T_3 (100 Gy) with the lowest plant height (65 cm). T_4 (150 Gy) produced the most fruits (40/plant in five months), while T_3 (100 Gy) had the highest yield (62.32 kg/plant). Family I had the earliest flowering (73 days), while Families P and E had the lowest first-flowering height (42 cm). Family O produced the most fruits, while Family I had the highest yield (63.59 kg/plant). Mutant R35P4, with a lower respiration rate, had the longest shelf life (14 days) among the M_1 mutants. R11P10, R11P8 and R3P4 (M_3 population) showed lower initial respiration rates, with peaks observed on the 10th day of storage. R35P4 showed ethylene peaks on the 13th day of storage. M_3 mutants R8P9, R11P8 and R3P4 had increased ethylene peaks on the 10th day. HRM primers were created for the ripening-related genes ACC synthase and ACC oxidase in order to amplify specific mutant samples using qPCR (RT-PCR) and investigate sequence variation. Samples showing shifts in peaks (about 25 samples) were selected and sanger-sequenced. Mutants such as R41P8, R38P19, R7P9, R16P6 and R39P20 showed insertions, deletions and substitutions for the ACC-Synthase gene. These results confirmed sequence variation due to mutation induction.

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Identifying the Genes Regulating Finger Number of Finger Millet (*Eleusine coracana*) under Drought Stress

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FINGER MILLET, a nutri-cereal grown in marginal soils, contributes to the food and nutritional security of Karnataka. Grains in cereals are formed from fertilized florets in inflorescence. Increasing floret number per spikelet or spikelet's per plant or regulating floret fertility are strategies to improve grain yield. Molecular mechanism underlying inflorescence meristem (IM) identity, maintenance and differentiation is obscure in finger millet with unique spike architecture, but well elucidated in rice, maize, wheat, barley and foxtail millet. In this study, attempt is made to understand the molecular mechanism regulating IM development (finger number) in finger millet using genotypes contrasting for finger number- HF (High finger) and LF (low finger), but having similar total dry matter. The genotypes were grown under drought stress (100, 60, 30% field capacity) to study the development of IM under drought stress. The development of IM was delayed under drought stress in both genotypes. Similarly, yield was reduced under drought stress in both LF and HF. The reduction of finger number per ear head was pronounced in HF compared to LF. The number of seeds per plant was significantly higher in HF compared to LF under control and drought stress. Transcriptomic analysis using RNAseq of HF (IM and leaves) grown under control and drought stress was carried out to identify the molecular regulators of IM development. The genes coding for transcription factors *viz., WRKY, MYB, MADS, AP2/B3, TCP, bHLH* and *bZIP* were found to be differentially regulated under stress, which probably are associated with stress tolerance. These TFs might be important for IM development and responsible for stress tolerance, which needs to be further validated. These genes could be possible targets for regulating finger number in finger millet.

Novel Multienzyme System Coupled with Hydrothermal Pretreatment for Enhancing Degradation of Bioorganic Substances

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BIOORGANIC waste, including agricultural and kitchen waste are generally managed by way of incineration, landfilling and biodegradation. Incineration and landfilling approaches are not environmentally sustainable and biodegradation is time-consuming. While in the biodegradation using microbes, the efficiency is lower due to recalcitrant nature of bioorganic substances as well as initial lag in microbial growth. Therefore, to address the initial lag and speed up the early degradation prior to log phase of microbial growth, physical and biochemical pretreatments were studied in different substrates such as cotton stalk, sugarcane thrash, potato peel and cucumber peel. The pretreatments were; physical, size reduction (1 mm, 0.7 mm, <0.5 mm) and hydrothermal (100 °C for 0.5-5 hours); chemical, ionic mixture (oxalic acid and sodium chloride at 0.2, 2, 5 and 10%); biochemical, cellulase and pectinase enzymes (both individual and combination). The matrix decomposition of substrate studied during hydrothermal treatment was found to be influenced by the particle size. In case of cotton stalk, particle size of 0.7 mm was found to be more appropriate were in the other substrates, maximum denaturation was observed below 0.5 mm. The selected powdered samples were subjected to different levels of ionic mixture treatment, in which 2 per cent oxalic acid and sodium chloride showed more matrix decomposition upon hydrothermal treatment. In the pretreated substances, both individual and combined enzyme treatments demonstrated enhanced degradation compared to the respective control in all substrates studied here. In contrast, the enzymatic activity in samples pretreated with ionic substances showed reduced effectiveness. Therefore, this study highlights the importance of physical and biochemical (different enzymes) pretreatment for maximizing the initial rate of biodegradation of bioorganic substances.

Green Synthesis of Zn and Fe Nanoparticles, its Formulation Using *Bacillus subtilis* Metabolites and its Effects on Maize

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DEFICIENCIES in iron, zinc, iodine and selenium, remains a significant health challenge in developing regions. Zinc (Zn) and Iron (Fe) deficiency, in particular, is a major risk factor for diseases like growth retardation and increased susceptibility to infections. Biofortification has emerged as a promising solution to address these deficiencies by increasing nutrient levels in staple crops such as rice, wheat and maize, which often grow in nutrient deficient soils. This study focused on synthesizing zinc and iron nanoparticles using hibiscus and eucalyptus leaves extracts and formulating nanoparticles with *Bacillus subtilis* (PHT1-21S) metabolites. The efficacy study was conducted on plant nutrient uptake and growth. Transmission electron microscopy revealed that zinc nanoparticles (ZnNPs) were mostly spherical, with a size ranging from 24-80 nm, while iron nanoparticles (FeNPs) had a more varied shape, with a size ranging from 40-90 nm. Scanning electron microscopy and energy-dispersive X-ray analysis confirmed the successful formation of zinc and iron oxides. Dynamic light scattering showed a narrow size distribution for both nanoparticles and Fourier-transform infrared spectroscopy identified functional groups involved in their synthesis. Nanoparticles efficacy study on maize demonstrated that treatment with 600 ppm of zinc or iron nanoparticles significantly improved growth parameters like plant height, cob length and kernel yield. Zinc nanoparticles at 600 ppm (T6) resulted in the highest cob weight (217.77 g), while zinc nanoparticles with *Bacillus subtilis* (T12) achieved the highest grain weight per cob (186.57 g). Biochemical analyses at 60 days after sowing showed that iron nanoparticles (400 and 600 ppm) enhanced chlorophyll content, while zinc nanoparticles (600 ppm) increased protein and soluble sugar content. Zinc and iron content in maize grains recorded was maximum in T6 (600 ppm Zn) and T9 (600 ppm Fe), respectively. The results demonstrated that nanoparticle- based biofortification could effectively improve maize growth, yield and nutritional quality, addressing micronutrient deficiencies.

Transcriptome Analysis of Rice Bean (*Vigna umbellata*) differing in Resistance to Yellow Mosaic Virus (YMV)

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RICE BEAN (*Vigna umbellata*), a leguminous crop known for its inherent resistance to viral diseases, serves as an ideal model to investigate molecular defense mechanisms against Mung bean Yellow Mosaic Virus (MYMV). This study aimed to identify rice bean accessions with varying resistance levels to MYMV and analyze transcriptional responses contributing to resistance. An agroinfection approach using *Agrobacterium tumefaciens* harboring full-length MYMV DNA-A and DNA-B was validated in a susceptible mung bean genotype (DGGV 2), confirming infectivity through mosaic symptom expression. Using the same method, 49 rice bean accessions were screened. Phenotypic evaluation revealed that 43 accessions as highly resistant, 3 resistant and 2 moderately resistant. For transcriptomic analysis, two contrasting accessions EC 33 (highly resistant) and IC 13 (moderately resistant) were selected. Leaf samples from control and infected plants were collected at 7, 15 and 30 days post-inoculation. RNA was extracted, quality-checked and subjected to mRNA purification and RNA-seq. High-quality libraries showed 73.99% - 78.88% alignment to the rice bean reference genome. Differential gene expression analysis revealed stronger post-infection defense activation in susceptible plants, while resistant plants showed tightly regulated expression of immune-related genes, including receptor-like kinases and R proteins. Down-regulation of calcium signaling and metabolic genes suggested energy reallocation during stress. Notably, constitutive expression of defense genes in uninfected resistant plants implied a preformed resistance mechanism. These findings enhance our understanding of MYMV resistance in rice bean and identify candidate genes for functional validation and breeding for viral resistance in legumes.

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Screening, Isolation and Cloning of Vip Gene from *Bacillus thuringiensis* Isolated from Soil Samples of Karnataka

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BACILLUS THURINGIENSIS (Bt) is a widely recognized microbial biopesticide that produces insecticidal proteins, notably Cry and Vip. Among these, Vip3A is known for its broad-spectrum insecticidal activity and distinct mode of action, making it a valuable tool in resistance management strategies. This study aimed to screen, isolate and clone the *vip3a* gene from native Bt isolates collected from soil samples across the Western Ghats and northern Karnataka. A total of 20 soil samples were processed using acetate enrichment, yielding 30 Bt isolates. These were screened for parasporal crystal formation and confirmed through 16S rRNA sequencing. SDS-PAGE analysis detected ~90 kDa Vip3A proteins in several isolates. Bioassays conducted against *Spodoptera litura* larvae revealed varying levels of insecticidal activity with isolate T19 showing the highest mortality. The *vip3a* gene (~2370 bp) was amplified from T19 and cloned into the pTZ57R/T vector, followed by transformation into *E. coli* DH5α and confirmed by sequencing. The gene was then subcloned into the pET28a expression vector and transformed into BL21 cells for protein expression. IPTG-induced expression was validated through SDS-PAGE analysis. These findings highlight the potential of native Bt isolates from Karnataka in the development of transgenic crops or biopesticide formulations for sustainable pest management.

Development and Validation of Core Sets in Grain Amaranth (*Amaranthus* spp.) Germplasm

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LARGE collection of germplasm poses difficulty for characterization and evaluation for target traits. To address such issues development of a reduced representative set popularly called as 'core set' is proposed. Towards this effort; the current work was under taken to assess the variability of 1730 germplasm collections representing different geographical regions. Substantial differences for qualitative traits and genetic variability for quantitative traits in the germplasm were evident from the study. A total of 08 core sets were developed using 08 quantitative traits data following standard stratified sampling strategies- based approaches. Qualitative traits-based statistics such as chi-square test and 'class coverage' and 'Shannon-Weaver diversity index' were used as validation criteria to examine homogeneity and retention of qualitative traits-based classes and polymorphism diversity, respectively in the core sets to assess their representativeness. Quantitative traits-based univariate statistics -mean, variance, range and multivariate statistics such as SMD%, CR%, VD% and VR% were used to assess the representativeness of core sets. Logarithmic strategy with a preferred sampling approach-based core set of ~10.0 percent size (180 entries) was identified as the best representative of the base collection. This core set is suggested to serve as a priority resource for use as working collection in breeding programme. To identify trait specific accessions from the core set, 180 grain amaranth germplasm accessions were characterized for 09 qualitative and 08 quantitative traits using augmented design at three locations *viz.,* Gandhi Krishi Vigyana Kendra (GKVK), Bengaluru; Zonal Agricultural Research Station (ZARS), Mandya and Agriculture Research Station (ARS), Arasikere during summer 2024. Based on inflorescence length (cm) and grain yield plant^{-1} (g) and volume weight of seeds (g/10ml) single trait specific and multiple traits specific accessions were identified. Ten accessions were found promising for combination of desirable traits.

Identification of SSR Markers Linked to Powdery Mildew Disease Resistance in Mungbean (*Vigna radiata* L. Wilczek)

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MUNGBEAN (*Vigna radiata* L. Wilczek) is an annual crop belonging to the family Leguminosae with 2n=2x=22. Mungbean production is adversely affected by powdery mildew disease caused by *Erysiphe polygoni* DC. Yield losses due to this disease were reported to be 20-40 per cent at reproductive stages. Thus, it is important to understand the genetics of powdery mildew disease resistance. Therefore, inheritance studies were undertaken using the cross involving Chinamung (Susceptible) × LM-1668 (Resistant). F_1s were resistant and F_2 plants segregated into 130 resistant: 50 susceptible. These data fitted well with the ratio of 3 resistant: 1 susceptible in Chinamung x LM-1668 indicating parents involved in the cross differed for single gene. The polymorphic survey was undertaken using 150 SSR markers of which eleven were polymorphic between parents and then subjected for bulked segregant analysis. Four SSR markers were found to be polymorphic between resistant and susceptible bulks and parents. Single marker analysis, significance of F-test, marker allelic frequencies, and their significance of t-statistic tested by two-sample t-test indicated two SSR markers (*DMBSSR125* and *CEDG044*) were putatively linked to powdery mildew disease resistant loci. Powdery mildew disease resistant loci were located at 2.30 cM distance from the marker *DMBSSR125* and at 9.30 cM distance from *CEDG044*. Both the SSR markers were confirmed in RIL population derived from Chinamung x BL849 and thus, found to be linked to powdery mildew disease loci in RIL population which was confirmed by single marker analysis, estimating marker allelic frequencies and their significance through two sample t-test. These markers can be utilized for marker assisted selection in breeding population. Five superior RILs (C1-1-3, C1-48-3, C1-89-5, C1-135-3 and C1-155-3) resistant to powdery mildew were identified based on their *per se* performance for seed yield. These superior RILs can be exploited in future mungbean breeding programmes.

Evaluation of Germplasm Accessions in Black Gram [*Vigna mungo* (L.) Hepper] for Grain Yield and Terminal Drought Stress under Field Conditions

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BLACK GRAM [*Vigna mungo* (L.) Hepper] is a highly valued pulse crop cultivated globally but often suffers significant yield losses due to biotic and abiotic stresses, particularly terminal moisture stress (TMS). Ninety six germplasm accessions, along with two checks (Rashmi and LBG791), were evaluated using an augmented design during the 2023 *kharif* season to identify high-yielding accessions under optimum growth conditions. These accessions were further assessed in an alpha lattice design during the 2023–2024 *rabi* season at the University of Agricultural Sciences, Bangalore to identify TMS-tolerant accessions under field conditions. Analysis of variance (ANOVA) revealed significant genetic variability for most traits, except for pod length. Traits such as pod yield and seed yield exhibited high broad-sense heritability and genotypic coefficient of variation (GCV), indicating limited environmental influence. K-means clustering grouped the accessions into nine clusters with inter cluster distances ranging from 3.53 to 6.37 D^2, revealing substantial genetic diversity. Four high yielding accessions identified for pod yield and seed yield plant^{-1} were AVUB-2017, TU-98-18, RU-16-20 and AVUB-2021. The germplasm accessions exhibited wide variability and strong positive correlations with seed yield under both non-stress (NS) and TMS conditions for all three traits in early and late flowering sets. Indices such as Arithmetic Mean Productivity (AMP), Geometric Mean Productivity (GMP), Harmonic Mean Productivity (HMP) and Stress Tolerance Index (STI) were effective for selecting TMS-tolerant accessions. High-yielding, TMS-tolerant accessions identified included AVUB-2020, RU-16-20, and TU-98-18 (early flowering), as well as MBG-5, VBN-9, UH-04-04 and CN-281987 (late flowering). These accessions offer significant potential for breeding programs aimed at enhancing drought resilience in black gram.

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Assessment of Genetic Diversity and Heterotic Grouping of Popcorn (*Zea mays* L. Var. *everta*) Inbred Lines

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An investigation was carried out to estimate the genetic diversity, combining ability, heterosis and heterotic grouping of popcorn (*Zea mays* L. var. *everta*) inbred lines for yield and quality traits. Fifty-three popcorn inbred lines were grouped into twelve clusters using Mahalanobis D^2 statistics. The cluster I accommodated maximum number of inbred lines (23) followed by cluster II (18). Combining ability analysis was performed using 53 lines and three testers by employing Line × Tester mating design. The ratio of GCA to SCA variance revealed the preponderance of non-additive gene action in the expression of all the traits except kernel rows per cob. The inbred line MPI 23-19 was the best overall general combiner for yield and quality traits followed by PIL 6-023 and MPI 23-08. The tester MRCPC-20 was identified as best general combiner for yield and quality traits in popcorn. Among crosses, PIL 6-020 × MRCPC-20, MPI 23-06 × BPCL-3 and PIL 6-017 × BPCL-6 exhibited highest significant SCA effects and high heterosis over checks for yield and quality traits. The cross MPI 23-12 × MRCPC-20 is the best specific combiner for popping volume followed by MPI 23-09 ×BPCL-3 and PIL 6-026×BPCL-3 along with higher heterosis over mid parent, better parent and standard checks. These hybrids need to be further evaluated across locations and seasons to select best hybrids for commercial exploitation. Heterotic grouping of inbreds was carried based on grain yield using the HSGCA method with three testers. With BPCL-6 and BPCL-3, 15 inbreds were grouped in A, 16 in B and 14 in AB group. Similarly, with BPCL-6 and MRCPC-20, 14 inbreds were grouped in B, 15 in C and 16 in BC group.

Characterization and Assessment of Genetic Variability among Backcross Derived Interspecific Inbred Lines in Sunflower (*Helianthus annuus* L.)

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Morphological characterization of any developed backcross derived interspecific inbred lines helps in developing a database based on which newly developed inbred lines can be identified and distinguished. Forty-nine backcross derived interspecific inbred lines and 15 checks were characterized for 26 qualitative traits and evaluated for 13 quantitative traits during the late *Rabi* season of 2024-25. The backcross derived interspecific inbred lines exhibited a wide range of morphological variability for most characters such as hypocotyl pigmentation; leaf size, shape, colour, number, blistering, serration and orientation; petiole pigmentation; angle between the lower part of the petiole and stem; stem pigmentation; ray floret shape, colour and number; disc floret colour; stigma pigmentation; pollen colour; days to 50 per cent flowering; bract shape; head attitude, diameter and shape; branching and its type; plant height; seed shape; seed coat base colour; seed stripes, colour of stripes and seed length; 100 seed weight; volume weight (g/100 ml); oil content; and hull content. The results of variability studies on these backcross derived interspecific inbred lines indicated the existence of substantial morphological variability for yield and yield attributing traits, as evidenced by PCV and GCV. Based on *per se* performance, the top ten backcross derived interspecific inbred lines were identified for each trait, such as seed yield per plant, oil content, days to 50 per cent flowering, and hull content. Based on overall ranking derived from multiple trait performance, ten superior lines were identified, combining earliness, high seed yield, higher oil content and reduced hull percentage. These promising lines hold potential as parental resources for hybrid development and for broadening the genetic base of sunflower.

Constitution of an Elite Breeding Panel for Multiple Traits in Rice (*Oryza sativa* L.)

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The current rice breeding programme faces challenges in combining multiple traits such as high yield, stress tolerance and grain quality within a single line. To address this, identification of elite lines is essential for integrating desirable traits into a line in an individual breeding programme. Out of 1488 genotypes evaluated, 121 genotypes selected based on high yield (>4 t/ha), resistance to leaf and neck blast and sheath blight and tolerance to BPH infestation (<5 SES, 2013) were further evaluated for yield and yield-attributing traits at Mandya and Gangavathi. Concurrently, they were also assessed for disease and pest resistance at Mandya, along with grain Zn and Fe content in dehusked grains. ANOVA revealed significant differences among genotypes across the two locations. Based on the evaluation, 41 genotypes were shortlisted for genotyping. Among these, four genotypes showed resistance to all the biotic stresses assessed (<5 SES) and were selected for inclusion in the Elite Breeding Panel (EBP). The 41 genotypes were genotyped using SSR markers for 53 genes associated with 15 essential traits. From this, seven genotypes were identified based on the highest number of desirable traits and nine genotypes were selected based on the highest number of favorable genes. Out of these 20 genotypes in total, eight elite genotypes (KMP-225, IET 29827, IET 30774, IET 29833, IET 29852, IET 30011, IET 29793 and IET 29800) were finalized for inclusion in the EBP. These genotypes represent promising resources for combining yield, multiple biotic stress resistance and nutritional quality in rice breeding programmes.

Test Cross Breeding Potential of Early Segregating Lines Derived from Three Bi-Parental Crosses in Castor (*Ricinus communis* L.)

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In traditional plant breeding, breeders perform large number of crosses every year to develop inbred lines. But due to resource constraints, such as longer duration (>6 months), wider spacing (120 cm × 60 cm) and higher labour required, makes it impractical for castor breeders to manage large populations in successive generations. Early generation testing is valuable because it saves time and resources by eliminating undesirable genotypes prior to the advanced generations. The parameters that are used to assess the breeding potential of the three bi-parental breeding populations (BP), JMJW, JIJM and JIDS. Trait means showed a consistent trend in favour of JMJW-derived test cross (TC) hybrids for most traits including yield component. Genotypic variance suggested greater breeding potential of JIDS BPs for mean seed yield plant^{-1}. Standardized range also indicated a trend favouring JIDS BPs for mean seed yield plant^{-1}. Transgressive segregation index and Usefulness index (UI) both identified JIDS BPs as superior for mean seed yield plant^{-1}. But favoured JMJW BPs for most yield-contributing traits in castor. Overall assessment of all breeding potential parameters, along with field observations, suggested that JMJW BPs are highly valuable for developing inbred lines. Under prediction hypothesis, TC hybrid potential (realized mean) of partial inbred lines derived from three bi-parental BPs and parental TC hybrids (predicted mean) were compared using two-sample 't'-test with unequal variance. Predicted and realized TC means were comparable for number of effective spikes with both the testers. Partial correspondence was also observed with tester DPC22 for total and effective spike length and mean seed yield plant^{-1}. Prediction and validation results for 100-seed weight matched only for JIDS BPs with both testers. Overall, the prediction results are highly reliable for those traits mentioned above due to absence of epistasis.

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Identification of Citronella Half-Sib Progenies (*Cymbopogon winterianus* Jowitt) with High Oil Content and Geraniol and Citronellal Ratio

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JAVA CITRONELLA (*Cymbopogon winterianus* Jowitt), an aromatic perennial grass highly valued for its essential oil rich in citronellal, geraniol and citronellol used in perfumery, cosmetics, pharmaceutical industries. However, limited genetic diversity, fluctuating oil yield and lack of improved varieties constrain its commercial cultivation. To overcome these challenges, the present investigation was undertaken to identify superior half-sib progenies with high oil yield and superior quality traits through polycross breeding. This study involved 60 half-sib progenies developed from four parents (CIM-Bio-13, Jal Pallavi, CIM-Jeeva and Medini) that were evaluated at CSIR–CIMAP Research Centre, Bengaluru, during *Kharif* and *Rabi* 2024–25. The experiment was laid out in a simple lattice design (8×8) with 2 replications and data were recorded on seven morphological and eight major oil constituent traits. Essential oil obtained by hydro-distillation was analysed in gas-chromatography. Significant morphological and biochemical variability was observed across the progenies, with several of them outperforming their parents for herb yield, oil content and biochemical composition. Cross-species transferability analysis was carried out using 124 SSR primers from *C. flexuosus*, 70 showed moderate transferability (56.45% amplification, 8 polymorphic), while of 17 primers from *C. citratus*, only 7 showed transferability (41.17% amplification, 2 polymorphic). Several superior progenies were identified, including B7 1-7 (highest herb yield: 1809.25 g/plant), B1 5-6 (highest oil content: 1.98%) and B25 6-10 (balanced GC ratio with high citronellal and geraniol). These results demonstrate that polycross breeding combined with molecular analysis can be a powerful tool to develop new citronella varieties with improved oil yield and quality traits.

Species Composition of Spider Mites on Major Vegetable and Flower Crops in the Eastern Dry Zone of Karnataka and Monitoring their Acaricide Resistance

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AN investigation was carried out to catalogue spider mite species infesting major vegetable and flower crops in the Eastern Dry Zone of Karnataka and to monitor the acaricide resistance of *Tetranychus urticae* Koch and *Tetranychus macfarlanei* Baker & Pritchard populations to fenazaquin, fenpyroximate and propargite. Field surveys across 68 locations in 15 taluks in the zone revealed 12 spider mite species: Ten species under the genus *Tetranychus* and one each under *Oligonychus* & *Eutetranychus*. The mites were recorded on 26 crops belonging to ten botanical families with *Tetranychus* spp. exhibiting the highest diversity. Four species were documented as new global host records: *Tetranychus fijiensis* Hikanzawarst on cucumber, *Eutetranychus orientalis* Klein on tomato, *T. macfarlanei* on the pointed gourd and *Tetranychus truncatus* Ehara on sponge gourd. Two new host records for India and five for Karnataka were also found. The highest diversity was recorded in the Ramanagara district with eight species. For resistance to fenazaquin, *T. urticae* population followed the order Ramanagara > Bengaluru Rural > Chikkaballapura (moderate to high resistance), for fenpyroximate the order was Ramanagara > Chikkaballapura > Bengaluru Rural (low to moderate resistance) and for propargite the order followed was Chikkaballapura > Bengaluru Rural > Ramanagara with all the populations showing a high level of resistance. For *T. macfarlanei* the resistance to fenazaquin was in the order of Bengaluru Urban > Bengaluru Rural > Ramanagara, with a low level of resistance and a similar low level of resistance was observed for fenpyroximate and propargite.

Exploration of Entomopathogenic Endophytes from *Simarouba glauca* against Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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ENDOPHYTES are the symbiotic microorganisms residing within plant tissues, enhance plant resistance to pests by producing phytohormones and bioactive compounds. The exploration of entomopathogenic endophytes from *Simarouba glauca* against *Spodoptera frugiperda* was studied using culture-dependent techniques based on ITS gene and 16S rRNA sequencing. Eight fungal and ten bacterial endophytes were isolated from the bark, leaves and seeds of *S. glauca* and characterized morphologically and molecularly. Among them, *Bacillus cereus* was found in all three parts of *S. glauca*, while *Bacillus subtilis* and *Penicillium citrinum* were belong to the bark and leaves. Fungal isolates belonged to the phylum Ascomycota. While 8 bacterial isolates were belong to the phylum Bacillota. Seven fungal isolates SBF1, SBF2, SBF3, SBF4, SLF2, SLF3 and SSF1 (1×10x conidia/ml) and seven bacterial isolates SBB1, SBB2, SBB3, SBB4, SLB1, SLB4 and SSB2 (6×10x cfu/ml) were screened for their effects on *S. frugiperda*. The fungal isolates significantly impacted the survival and development of *S. frugiperda*. Among them, SBF1-*Cladosporium perangustum* and SBF2-*Diaporthe hongkongensis* exhibited the highest larval mortality (35.15 and 24.91%, respectively). Additionally, fungal treatments prolonged larval and pupal durations and reduced larval and pupal weights. *Cladosporium perangustum* notably reduced pupation (73.33%) and adult emergence (72.86%), while also decreasing female adult longevity and fecundity. Egg hatchability was drastically reduced in larvae treated with SBF3-*Penicillium citrinum*. Bacterial isolates, particularly SBB2-*Bacillus subtilis* and SSB2-*Enterobacter hormaechei*, caused significant larval mortality (24.14 and 17.24%, respectively). *B. subtilis* notably prolonged developmental periods, reduced pupation rates (76.67%) and affected adult emergence (78.57%).

Host Plant Resistance in Tomato against Major Insect Pests

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THE studies on host plant resistance in tomato against major insect pests was conducted during *Rabi* and *Summer* 2023-24 at the College of Agriculture, Hassan to evaluate fifteen tomato genotypes (including ARKA RAKSHAK as check) under open field condition for resistance against major insect pests *viz*., leaf miner (*Liriomyza trifolii*), pin worm (*Phthorimaea absoluta*) and fruit borer (*Helicoverpa armigera*) and analyzed the morphological and biochemical traits attributed to resistance. Peak infestation of pests was observed at 90-120 days after transplanting (DAT). The genotypes 2019TODVAR6 and 2019TODVAR10 consistently exhibited strong resistance against the pests. While, the genotypes 2019TODVAR5, 2014TODVAR3 and 2019TODVAR9 displayed high susceptibility in comparsion to check ARKA RAKSHAK. Among the morphological parameters, trichome density displayed significantly positive correlation with pest resistance. The genotype, 2019TODVAR10 (26.96 trichomes/cm²) showed higher trichome density whereas, genotype 2019TODVAR5 (16.48 trichomes/cm²) exhibited least trichome density, harboured highest and lowest pest incidence, respectively. Among the biochemical parameters, total phenols displayed significant positive correlation, while total sugars displayed significant negative correlation to pest resistance. Among the genotypes, 2019TODVAR4 (4.69 mg/g.fr.wt) recorded highest total phenols and lowest total sugars (3.49 mg/g.fr.wt) harboured lesser pest incidence. On contrary, the genotype 2019TODVAR5 (2.40 mg/g.fr.wt) with lowest total phenols and highest total sugars (5.69 mg/g.fr.wt) noticed lesser pest incidence. In nutshell, the genotypes 2019TODVAR6, 2019TODVAR4 and 2019TODVAR10 were categorised as promising genotypes for breeding pest resistant tomatoes, emphasizing the importance of morphological and biochemical parameters as key resistance traits. These findings will provide valuable insights for developing sustainable pest management strategies and improving tomato crop resilience.

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Insect and Mite Pests of Rose in the Eastern Dry Zone of Karnataka and their Management

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A study was conducted on insect and mite pests of rose in the Eastern Dry Zone of Karnataka and their management from January to July, 2024. During the survey, eight thrips species- *Scirtothrips dorsalis* Hood, *Frankliniella schultzei* (Trybom), *Megalurothrips typicus* (Bagnall), *Thrips parvispinus* (Karny), *Thrips palmi* (Karny), *Thrips florum* (Schmutz), *Rhipiphorothrips cruentatus* Hood and *Exothrips hemavarna* (Ramakrishna & Margabandhu); three aphid species- *Macrosiphum rosae* (L.), *Aphis craccivora* Koch and *Myzus persicae* (Sulzer); three species of foliage feeders- *Myllocerus viridanus* (Fabricius), *Euproctis fraterna* (Moore) and *Cytacanthacris tatarica* (L.); four speices of foliage & flower feeders- *Popillia schizonycha* (Arrow), *Gametis versicolor* (Fabricus), *Helicoverpa armigera* (Hubner) and *Achaea janata* (L.); four species of spider mites- *Tetranychus urticae* Koch, *Tetranychus neocaledonicus* Andre*, Tetranychus ludeni* Zacher and *Oligonychus biharensis* (Hirst) were recorded. In May, thrips populations peaked and it was lowest in February. Mites population was most abundant in January, declining thereafter. Bud borer infestation was highest in January and the aphid infestation was peak in February and other insect pests were predominant in June. The tapping method proved more effective for sampling of thrips than the bud dipping method. Among pest control treatments, T3, T4 and T5 (combinations of synthetic pesticides, botanicals, entomopathogenic fungi and natural oil) were most effective in reducing the thrips by 73.56, 76.22 and 74.45%, respectively. T6 (botanicals, entomopathogenic fungi and natural oil) was the least effective with 57.31 per cent reduction in mites. Cost analysis showed T3 and T5 as superior treatments, costing INR 13,610 and INR 13,670 per hectare, respectively.

Studies on Insect Pests of Little Millet and Management of Shoot Fly

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Study was conducted to know the insect pests and its population dynamics, resistance screening and management of shoot fly, *Atherigona pulla* (Wiedemann) in little millet. Insect pests such as weevils, grasshoppers, aphids and natural enemies like coccinellids and spiders were recorded. During *kharif* 2023, shoot fly infestation was peaked at 31.11 per cent in mid-September and in *kharif* 2024, it was reached 38.89 per cent in mid-July. Natural enemies were active in both season with coccinellids and spider population peak at mid-September (2023) and July(2024). Screening of little millet germplasm revealed significant variability in resistance to shoot fly. Highly resistant genotypes are GPMP 609, GPMP 675, OLM 203 and GPMP 728 exhibited low per cent dead-heart (5.00-6.67%), while highly susceptible genotypes are GPMP 745, GPMP 700, GPMP 607 and DPLV 1 showed high infestation levels (53.33-55%). Resistant genotypes had higher phenol (0.71-0.76%) and potassium content (0.50-0.53%) negatively correlated with shoot fly incidence. Conversely, susceptible genotypes had higher sugar (0.11-0.12%), protein (2.95-3.06%), nitrogen and phosphorus levels, all positively correlated with infestation. Among management strategies, imidacloprid 48FS @ 12 ml/kg of seeds (T_1) was most effective, reducing dead-heart incidence by 44.68 per cent (2023) and 54.09 per cent (2024). Cyantraniliprole19.8%+thiamethoxam 19.8%FS @ 4 ml/kg of seeds (T_3) with reductions over control of 39.35 (2023) and 46.72 (2024) per cent and lamdacyhalothrin 5% EC @ 1 ml/l (T_6) were also effective. The study revealed importance of resistant germplasm, phenol, potassium content and effective chemical management for minimizing shoot fly damage in little millet.

Survey, Screening and Biochemical Factors Mediated Resistance in Rice against Brown Planthopper, *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae)

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The present investigation was carried out on survey, screening, biochemical factors mediated resistance and identification of major QTLs for brown planthopper *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae) tolerance in rice. Among different locations surveyed in three districts of Southern Karnataka for the incidence of brown planthopper during *kharif* 2023, the highest number of hoppers were found in T. Narsipura taluk (27.86 hoppers/hill), followed by Kollegal (24.08 hoppers/hill) and least numbers were found in Maddur (12.30 hoppers/hill). Among the 258 rice landraces and 705 genotypes screened against BPH in glasshouse, none of them were found to be highly resistant. Few were resistant and moderately resistant, while majority of them showed susceptible and highly susceptible reaction. A molecular characterization of fifty rice genotypes was performed using eight SSR markers associated with five BPH resistance genes that include *Bph3*, *Bph32*, *Bph17*, *Bph6* and *Bph31*. The analysis revealed that the genotype KMR-3 contained all five resistance genes and recorded a phenotypic score of 3 indicating moderate resistance to BPH infestation. Likewise, the studies on biochemical basis of resistance among the selected rice landraces and genotypes at 30 DAT against BPH revealed that, total soluble sugars, reducing sugars, crude proteins and nitrogen were found to be high in susceptible genotypes and were positively correlated with BPH incidence. In contrast, higher amounts of total phenols, tannins, total free amino acids, phosphorus, potassium and silicon were observed in all the resistant genotypes and exhibited negative association with BPH incidence.

Annotation, Identification and Characterization of Important Gene Families from *De Novo* Transcriptome of Cassava Mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae)

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The cassava mealybug (CMB), *Phenacoccus manihoti* Matile-Ferrero is a highly destructive invasive pest that poses a significant threat to cassava production. Current management strategies including chemical and biological controls face challenges such as pesticide resistance and the potential for hyperparasitoid interference. A comprehensive genetic diversity and geographical distribution assessment was conducted using 20 samples collected from Tamil Nadu and Kerala. Analysis of the mitochondrial cytochrome C oxidase subunit I (*mtCO1*) gene (average length: 503 bp) of the population revealed low genetic diversity likely due to thelytokous parthenogenesis with four haplotypes identified. Phylogenetic analysis using the Neighbour-joining method with Tamura-3 parameter revealed the *P. manihoti* population was divided into two distinct clades, with genetic distances ranging from 0.0000 to 0.0041 per cent. *De novo* transcriptome sequencing was performed on crawler and adult stages to investigate molecular mechanisms behind important traits. High-quality reads were assembled using Trinity and annotated *via* multiple databases. Differential expression analysis identified 14,773 differentially expressed genes (DEGs). Among these, 55 cytochrome P450 (*CYP450*) genes were identified and classified into 17 families and 20 subfamilies with four novel subfamilies discovered (*CYP7IGU, CYP87M, CYP706AL* and *CYP706AM*). Quantitative real-time PCR of ten selected *CYP450* genes showed notable expression differences between crawlers and adults. This study highlights the low genetic diversity of *P. manihoti* populations and provides high-quality transcriptomic data, offering insights into its biology and adaptations. Future research should explore genetic mechanisms driving its invasiveness and evaluate *CYP450* genes as potential targets for sustainable pest management strategies such as RNAi or CRISPR-based approaches.

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Survey on the Incidence, Pest Status, Source of Resistance and Management of Paddy Earhead Bug, *Leptocorisa oratorius* (Fab.) (Alydidae: Hemiptera) in Cauvery Command Area

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The investigations on survey on the incidence, varietal response, mechanism of resistance and management of earhead bug through bio-pesticides in paddy was undertaken at College of Agriculture, V. C. Farm, Mandya during 2023-24. Among different locations in three districts of southern parts of Karnataka surveyed for the incidence of paddy earhead bug during *kharif* 2023 and summer 2024, the highest per cent grain damage was recorded in T. Narsipura and least was observed in Maddur. During *kharif* 2023, the peak incidence of earhead bug was noticed in last week of November. The maximum temperature exerted a significant negative association while, afternoon relative humidity and sunshine hours exerted significant positive association with population of earhead bug. Similarly, minimum temperature, rainfall and number of rainy days exerted non-significant negative association. While, morning relative humidity had non-significant positive association with population of ear head bug. Among the 252 landraces of paddy evaluated against earhead bug resulted in identifying as 10 resistant, 65 moderately resistant, 118 moderately susceptible, 53 susceptible and 6 highly susceptible landraces. Among the morphological characters, plant height, number of panicles/plant, length of panicle showed non-significant negative association, while grain length, width and thickness showed significant positive association. Among the various biochemical constituents in paddy, the crude proteins, total soluble and reducing sugars, nitrogen and phosphorous showed a significant positive association while, total phenols, tannins, total free amino acids and potassium showed a significant negative association with ear head bug infestation. Among bio-pesticides evaluated against earhead bug, *L. saksenae* and *L. lecanii* @ 5.00 g L^{-1} were found effective and recorded higher grain yield, net returns and B:C ratio (1:2.59 and 1:2.53) compared to other treatments.

Studies on Insect Pests of Browntop Millet and Management of Shoot Fly

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A study was conducted to know the insect pests and its population dynamics, resistance screening and management of shoot fly [*Atherigona approximata* (Mollach)] in browntop millet. Insect pests on browntop millet such as weevils, grasshoppers, aphids and natural enemies like coccinellids and spiders were recorded. During *kharif* 2023, shoot fly infestation was peaked at 30.25 per cent in mid-September, while during *kharif* 2024, it was reached 48.23 per cent in mid-July. Natural enemies were active throughout both seasons with coccinellids and spiders showing population peaks in mid-September (2023) and mid-July (2024). Screening of browntop millet germplasm revealed significant variability in resistance to shoot fly. Highly resistant genotypes are VBT, 004D, GPUBT 5 and TNAU 128C exhibited low per cent dead-heart (8.33-10.0), while highly susceptible genotypes are GPUBT 1, GPUBT 4D and TNAU 123D showed high infestation levels (50.0-51.67). Resistant genotypes had higher phenol (0.41–0.43%) and potassium content (0.53–0.56%), negatively correlated with shoot fly incidence. Conversely, susceptible genotypes had higher sugar (0.11–0.12%), protein (3.01–3.12%), nitrogen and phosphorus levels, all positively correlated with infestation. Among management strategies, thiamethoxam 30 FS @ 10 ml/kg seeds (T_2) was most effective, reducing dead-heart incidence by 50.0 per cent (2023) and 55.55 per cent (2024). Cyantraniliprole + thiamethoxam FS (T_3) with reductions over control of 45.20 (2023) and 48.41(2024) per cent and lambda-cyhalothrin 5% EC (T_6) were also effective. The study revealed that the importance of resistant germplasm, phenol, potassium content and effective chemical management for minimizing shoot fly damage in browntop millet.

Reaction of Chilli Hybrids Against Black Thrips, *Thrips parvispinus* (Karny) with Special Reference to Incidence, Mechanism of Resistance and its Management through New Insecticides

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THE investigations on reaction of chilli hybrids against black thrips, *Thrips parvispinus* (Karny) with special reference to incidence, mechanism of resistance and its management through new insecticides were carried out at the Department of Agricultural Entomology and 'A' Block, College of Agriculture, V.C. Farm, Mandya, Karnataka during *rabi* 2023 and *summer* 2024. The field evaluation of thirty-two chilli hybrids along with susceptible check (Byadagi Kaddi) was screened against black thrips resulted in identifying three resistant and two moderately resistant chilli hybrids. Further, Among the biophysical characters *viz.,* plant height, plant spread, number of primary branches per plant, number of fruits per plant and length of pedicle showed significant negative association with black thrips infestation. Whereas, the biochemicals *viz.,* total free amino acids, tannins and phenols were negatively associated with per cent leaf curl incidence. While, total soluble sugars, total reducing sugars and crude protein were found positively correlated with per cent leaf curl incidence. Among the nutrient factors, nitrogen was found to be positively correlated with per cent leaf curl incidence. Whereas, phosphorous and potassium showed a negative association. Among seven insecticides evaluated fluxametamide 10 EC @ 0.75 ml/l, broflanilide 20 SC @ 0.10 ml/l and imidacloprid 17.8 SL @ 0.30 ml/l were found highly effective against chilli black thrips. However, fluxametamide 10 EC @ 0.75 ml/l was found superior in recording higher fruit yield, net profit and B: C ratio compared to other treatments.

Studies on Fungal Leaf Spots and Blights in Medicinal and Aromatic Crops

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MEDICINAL and aromatic crops are vital to the pharmaceutical, perfumery and cosmetic industries, yet their production faces significant threats from under explored fungal leaf spots and blights. Addressing this critical gap, research conducted from 2021 to 2024 at the University of Agricultural Sciences, GKVK, Bengaluru, focused on characterising and identifying the major fungal pathogens causing leaf spots and blights and devising effective management strategies. A purposive sampling survey was conducted in 2022 across Bengaluru. Symptomatology studies revealed distinct progression patterns for each disease. Isolation and purification yielded 27 fungal isolates. The pathogenicity of all isolates was proved by establishing Koch's postulates. Morpho-molecular characterisation of 11 selected virulent isolates unveiled 10 pathogens as first report in India: *Alternaria alternata* (*A. vera*), *Alternaria alternata* (*S. rosmarinus*), *Beltrania rhombica* (*P. graveolens*), *Bipolaris heveae* (*A. calamus*), *Colletotrichum plurivorum* (*C. quadrangularis*), *Colletotrichum sydowii* (*T. cordifolia*), *Curvularia geniculata* (*C. cuspidatus*), *Curvularia spicifera* (*W. somnifera*), *Exserohilum rostratum* (*C. flexuosus*), *Fusarium equiseti* (*S. rebaudiana*), and *Neopestalotiopsis clavispora* (*P. amaryllifolius*). Cultural studies demonstrated varying media preferences across the pathogens for optimal growth and sporulation, while physiological studies highlighted optimal growth conditions at 25°C–30°C and a pH of 6-7. Triazole fungicides were most effective *in vitro*, alongside potent antifungal activity from garlic and neem botanicals. Biocontrol agents, *Trichoderma* spp. and *Pseudomonas fluorescens*-M exhibited robust antagonistic effects, corroborated by GC-MS analysis of their volatile organic compounds. A pot culture experiment on rosemary infected with *Alternaria alternata* revealed propiconazole as the most effective fungicide. *P. fluorescens*-M significantly boosted plant defence enzyme (PAL, PPO, SOD and POX) activities in rosemary plants challenged with *Alternaria alternata*.

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Unravelling Pathogenicity and Virulence Strategies of *Magnaporthe grisea* Inciting Finger Millet Blast by Multifarious Approach

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Finger millet blast caused by *Magnaporthe grisea* is a major threat to global finger millet production, affecting all growth stages and causing significant yield losses. This study investigates the diversity, virulence and pathogenic strategies of *M. grisea* isolates through morphological, histopathological, biochemical and genomic analyses. Morphological characterization of 28 isolates revealed considerable variability in colony texture, colour and growth (5.90-8.90 cm), with conidial counts ranging from 1.30 to 39.75 per microscopic field. Oat meal agar was the most suitable medium for growth and sporulation. *ITS*-based molecular characterization identified 19 haplotypes with Haplotype 5 predominating, while mating-type analysis showed the presence of male (60.72%), female (25.00%), hermaphrodites (7.14%) and isolates of unknown fertility (7.14%). Virulence-associated gene analysis indicated the presence of *AGL* and *APS* genes in all isolates, while the *PWL* gene was detected in FMP 68. *AVR-Pik* and *AVR-Pia* were absent in all isolates. Six pathotypic groups were identified based on pathogenicity based pathotyping, with Pathotype IV being the most virulent. Field screening of 34 advanced genotypes across six locations revealed varying resistance levels with WN-562, DHFM-9-5, RAuF-17, VL-410, KOPN-1056 and VR-1163 showing resistance to leaf, neck and finger blast. Greenhouse studies confirmed WN-562's high resistance to Pathotypic group II and VR-384, VL-400 and KMR-204 as resistant across all pathotypic groups. Histopathological analysis highlighted a hypersensitive response in resistant genotypes, restricting fungal spread, whereas susceptible genotypes facilitated pathogen colonization. LC-MS/MS analysis detected mycotoxins such as Trichodermin, Pyrichalasin H derivatives and Pyriculol, enhancing pathogenicity. Whole-genome sequencing revealed a 41.76 Mb genome with 10,173 protein sequences and enriched carbohydrate metabolism pathways. Comparative genomics confirmed close phylogenetic relationship of *M. grisea* infecting finger millet with isolates from *Eleusine coracana*, *Eleusine indica* and *Oryza sativa*. These findings provide critical insights into virulence mechanisms and resistance strategies, enabling effective disease management and resistant cultivar development.

Studies on Powdery Mildew Pathogen Infecting Cucumber (*Cucumis sativus* L.) with Reference to its Occurrence, Characterization and Management

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Powdery mildew, an obligate biotrophic fungal disease, affects cucurbits, causing direct and indirect losses. It appears as white, powdery patches on leaves, stems and fruits, leading to yellowing and defoliation. A survey in districts of Bangalore, Chikkballapur, Doddaballapur, Kolar collected 42 isolates from cucurbits, including cucumber, snake gourd, bottle gourd, pumpkin, ridge gourd, ash gourd, ivy gourd, zucchini, sponge gourd and bitter gourd. The Percentage Disease Index (PDI) ranged from 32.42 to 97.78 per cent, indicating widespread infection with cucumber showing the highest severity at Bagalur. Morphological and molecular analysis (ITS and species-specific primers S1/S2, G1/G2, L1/L2) identified *Podosphaera xanthii* as the predominant pathogen, while pumpkin, ash gourd, sponge gourd and ivy gourd were infected by *Golovinomyces cichoracearum*. Cucumber genotypes were evaluated under polyhouse and field conditions during *rabi, kharif* and *zaid* seasons. Disease progression analysis showed Arka Veera and Bengaluru local variety exhibited slower infection rates and higher resistance, while VRCU-2246, VRCU-2210 were highly susceptible. Biochemical profiling of resistant (Arka Veera) and susceptible (VRCU-2246) genotypes revealed higher enzyme activity in resistant plants, peaking at 48h (PPO, PAL), 24h (PO) and 12h (SOD). LCMS-based metabolite profiling identified 1,459 metabolites, 313 unique to susceptible and 244 to resistant genotypes. Resistance was linked to purine, methionine and beta-alanine metabolism, with key defense compounds including alkaloids, fatty acyls and carboxylic acids. *In vitro* evaluations of botanicals and biostimulants against *P. xanthii* on cucumber showed *Ascophyllum nodosum* (63.37%) was most effective in suppressing disease. Bioagents and biosynthesized nanoparticles were also tested with Bio-AgNP at 1 per cent showing highest efficacy (69.49%). *In vivo* experiments confirmed *Ampelomyces quisqualis* (58.54%), *Bacillus subtilis* (56.23%) and *A. nodosum* (57.05%) effectively reduced disease severity with Propiconazole (25% EC) (58.60%) being most effective. This study provides insights into host resistance, pathogen diversity and eco-friendly management strategies for powdery mildew in cucurbits.

Characterization of Effector Proteins of Hemi-Biotrophic Pathogen *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Causing Mango Anthracnose and Understanding the Switch from Biotrophy to Necrotrophy

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Mango, a vital fruit crop, is severely affected by anthracnose, a postharvest disease that reduces fruit quality, shelf life and marketability. Twenty-nine isolates of *Colletotrichum* spp. were collected from nine states in India. Multigene analysis of the sequences of ITS, ACT, CAL, GAPDH and TUB2 genes revealed that *Colletotrichum asianum* (86 %) and *C. siamense* (24 %) were the predominant species. Pathogenicity tests confirmed that all isolates were pathogenic and caused symptoms on artificially wounded leaves and fruits of mango. *C. asianum* was used to screen mango genotypes for anthracnose resistance in 2022 and 2023 through artificial inoculation. Lesion diameters on leaves ranged from 0.03 to 1.73 cm, with inflorescence discoloration ranging from 14 to 56 per cent. Fruit lesion size ranged from 0.68 to 3.91 cm. After two years of screening, the wild species *M. odorata* and genotype *viz*., Prior and Gomavu were identified to be moderately resistant. Defense-related enzyme activities were analyzed in 'Prior' (MR) and 'Kensington' (HS) after *C. asianum* infection. Higher chitinase and β-1,3-glucanase activities in 'Prior' suggested their role in fungal cell wall degradation and disease resistance. Salicylic acid significantly inhibited mycelial growth and spore germination in a concentration-dependent manner. Gene expression analysis for fruits of 'Gomavu' (MR) and 'Banganapalli' (S) treated with 2mM SA, identified MRNBS11, PR1A, PR4, PR5, PAL and POX genes as key contributors for defense response against *C. asianum*. Transcriptomic analysis of Prior and Kensington revealed the expression of pathogenicity-related effector genes at different infection stages, which interact with RLPs, RLKs and R genes, activating signal transduction through kinases such as CDPK and MAPK. This inturn, triggered the SA, JA and phenylpropanoid pathway, leading to the activation of defense enzymes. These findings provide valuable insights for breeding resistant cultivars and developing effective disease management strategies.

Molecular Characterization of Cucumber Green Mottle Mosaic Virus Infecting Cucumber

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Cucumber green mottle mosaic virus (CGMMV), a major *Tobamovirus* infecting cucurbits, poses a serious threat to global cucumber production. To assess the prevalence and impact of CGMMV, survey was conducted in major cucumber-growing regions of Karnataka during 2023-24. Symptoms, including mosaic mottling, leaf distortion, blistering and fruit deformities were observed. The highest disease incidence was observed in Kolar (40.34%), followed by Bengaluru Rural (36.32%) and Chikkaballapura (35.62%). Molecular detection *via* RT-PCR confirmed CGMMV infection in 19 of 32 symptomatic samples collected from the farmers fields. From 16 samples, sequencing of the coat protein gene revealed high nucleotide identity (96.22–99.49%) with global isolates. Phylogenetic analysis revealed the grouping of Karnataka isolates into distinct clades, indicating international movement and multiple introduction events. Haplotype network analysis identified five populations, with dominant clusters in Ramanagara, Kolar, Chintamani and smaller populations in Devanahalli and Chikkaballapura, suggesting recent introductions of the virus isolates. Moderate nucleotide diversity ($\pi = 0.0092$) and Tajima's D value (1.4673) indicated balancing selection maintaining viral variants. The study also evaluated CGMMV's host range, showing differential symptom expression on indicator hosts and infection predominantly in cucurbits. To investigate the differential phytohormonal responses in CGMMV-infected cucumber plants, phytohormones were quantified at various time points, which revealed dynamic changes. Early increase in salicylic acid and jasmonic acid levels was followed by their suppression indicating disruption of defense pathways. While altered levels of gibberellic acid, indole-3-acetic acid and benzyl aminopurine highlighted the virus's manipulation of growth processes. These findings provide critical insights into CGMMV's genetic diversity, host specificity and hormonal dynamics, underscoring the urgent need for targeted management strategies to protect cucumber cultivation.

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Green Synthesis of Silica Nano Particles and its Management in Application of Early Blight of Tomato

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THE mycosynthesis of nanoparticles is an emerging eco-friendly approach that aligns with the United Nations Sustainable Development Goals (SDGs). This study focuses on the biosynthesis of silica nanoparticles (SiNPs) using *Fusarium oxysporum* and *Aspergillus niger*, isolated from agricultural wastes *i.e.,* rice husk and maize cob. The fungal isolates were identified supported by morphological, cultural and molecular characterization and their ITS sequences are submitted to the NCBI database (Accession: PQ423909 for *F. oxysporum* and PQ425658 for *A. niger*). Silica nanoparticles were synthesized through fungal-mediated biogenic processes and characterized through UV-Visible spectrophotometry, dynamic light scattering (DLS), zeta potential analysis and transmission electron microscopy (TEM). The synthesized SiNPs exhibited a surface plasmon resonance peak between 285–315 nm, with an average particle size of 51.47 nm (*F. oxysporum*) and 72.99 nm (*A. niger*). TEM analysis revealed spherical nanoparticles with a size range of 11.54–38.08 nm. The zeta potential analysis confirmed stable dispersion with distinct peak at -2.08 mV with *F. oxysporum* and -15.4 mV with *A. niger* -mediated SiNPs demonstrating superior dispersibility.The antifungal potential of SiNPs was evaluated against *Alternaria solani*, the causative agent of early blight in tomatoes, using *in vitro* and *in vivo* assays. At 100 ppm, SiNPs exhibited 99.39% mycelial growth inhibition in the poisoned food technique, while detached leaf assays recorded 84.81 and 83.99 per cent lesion inhibition in pre- and post-inoculation treatments, respectively. Greenhouse trials demonstrated reduction in disease intensity by 52.93 per cent with 100 ppm SiNPs comparable to commercial SiNPs (83.65%) and Mancozeb (95.88%). However, commercial SiNPs exhibited phytotoxic effects in pot trials. The findings establish the potential of mycosynthesized SiNPs as an alternative to chemical fungicides, offering a sustainable strategy for plant disease management. This study provides a basis for future research on fungal-mediated nanoparticle synthesis and its application in crop protection.

Studies on Host Plant Resistance and Integrated Disease Management of Fusarium Stalk Rot of Maize Incited by *Fusarium verticillioides* (Sacc.) Nirenberg

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FUSARIUM stalk rot caused *by Fusarium verticillioides* is a significant post-flowering stalk rot disease of maize. A comprehensive investigation was carried out involving disease survey, screening of inbred lines and hybrids, assessment of genetic diversity among isolates and integrated disease management strategies against FSR. A roving survey was conducted across nine major maize-growing districts of southern Karnataka. The survey results revealed highest disease incidence in Chitradurga district (45.5%) and the lowest in Mysore district (10.5%). A total of 135 inbred lines and 67 hybrids were screened against FSR through artificial inoculation. Out of these, six inbred lines (MAI-295-1, MAI-138-1, MAI-E2-105, MAI-E2-47, MAI-1-3, MAI-E2-135) and five hybrids (MAI-20-40, VL-15192XCML-581, HQPM-1, MAH-23-38, MAH-23-41) exhibited a resistant reaction with a disease score of 1.67 and 1.75. The pathogen was isolated from collected surveyed samples, purified and identified based on cultural and morphological characteristics and confirmed by PCR using species-specific primers (Actin, β-tubulin, and EF). Fifteen isolates were confirmed as *F. verticillioides* among which FV9 and FV11 were distinct species. UPGMA-based dendrogram analysis grouped the isolates into four main Clusters, Cluster IV had 11 isolates, Cluster III had 3, Cluster II had 2, and Cluster I had 1. Maximum genetic diversity was observed between FV1 and FV2 and minimum diversity between FV1 and FV9. Under *in vitro* evaluation of fungicides, botanicals and bioagents against Mandya isolate of *F. verticillioides*, the fungicide Carbendazim 50 WP (99.15%), plant extract Neem seed (99.99%) and bioagent *Trichoderma viride* (74.30%) showed maximum per cent growth inhibition of *F. verticillioides.* In field conditions T_{15} (seed treatment with Carbendazim 50 WP @ 0.2% + *T. viride* @ 1.0 % + soil application of neem cake @ 200 kg/acre + double dose of MOP @ 2 %) was most effective, showing the lowest PDI (21.3) and highest PDEC (71.7).

Exploring the Diversity of Seed-Borne Pathogens Infecting Tomato and Chilli Crops

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Tomato (*Solanum lycopersicum* L.) and chilli (*Capsicum annuum* L.) are economically important crops in Karnataka, where seed-borne pathogens significantly impact their productivity. A comprehensive study on survey, isolation, molecular detection and characterization of seed-borne pathogens revealed that the incidence of anthracnose of chilli was ranging from 35 to 80 per cent, with the highest incidence recorded at Kolar (67.08 %). However, the incidence of early blight of tomato disease was highest in Chikkaballapura (up to 61 %). The bacterial disease *viz.*, bacterial leaf spot and bacterial speck incidence showed 54.0 to 72.20 per cent and 52.0 to 54.0 per cent, respectively. Similarly, the incidence of tomato leaf curl virus (ToLCV) and chilli leaf curl virus (ChiLCV), were highly prevalent in Kolar recorded 76.35 and 72.22 per cent, respectively. Seed-borne fungal pathogens *viz*., *Colletotrichum truncatum* and *Alternaria alternata* isolated using ISTA agar plate method, were identified based on colony morphology, conidial structure and ITS region sequencing. Molecular characterization of *C. truncatum* (Ct-01 isolate) was further validated using actin and β-tubulin genes, while *A. alternata* (Alt-01) was confirmed using species-specific primers (Alt a1 and AA). Seed borne bacterial pathogens *viz.*, *Xanthomonas euvesicatoria* and *Pseudomonas syringae* pv. *tomato* were isolated on nutrient agar and were confirmed through 16S rRNA sequence analysis. Although ToLCV and ChiLCV were detected in symptomatic leaf samples, PCR analysis of seeds collected from ToLCV and ChiLCV infected plants confirmed that these viruses are not seed transmitted. The present study highlighted on importance of using pathogen-free seeds and adopting integrated disease management strategies to control seed-borne diseases in tomato and chilli crops.

Investigation on Novel Strategies for Mitigating Finger Millet [*Eleusine coracana* (L.) Gaertn] Blast Incited by *Magnaporthe grisea*

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Finger millet [*Eleusine coracana* (L.) Gaertn] suffers significant yield losses due to blast disease caused by *Magnaporthe grisea*. In present study, fifteen blast samples were collected from five Indian states and *M. grisea* was isolated via spore drop technique and confirmed through morphological and molecular characterization. *In vitro* screening revealed that biosynthesized silver nanoparticles ($AgNO_3$ NP) at 200 ppm exhibited the highest inhibition (67.46 %), surpassing the fungicide tricyclazole 18% + (61.26 %). Among seaweed extracts, *Ascophyllum nodosum* showed 37.78 per cent inhibition at 5 per cent concentration. *Trichoderma* species demonstrated complete *in vitro* inhibition, while *Bacillus velezensis* and *Pseudomonas fluorescens* showed 79.67 and 45.95 per cent inhibition, respectively. Greenhouse trials indicated that seed treatment with *Trichoderma viride* @ 10 g/kg + foliar application of *Trichoderma viride* @ 10 g/L resulted in the lowest disease intensity (20.74 %) and significant growth improvements. Field evaluations showed that seed treatment with AMC @ 10 g/kg + foliar application of biosynthesized $AgNO_3$ NP @ 100 ppm was most effective against leaf blast, while seed treatment with AMC @ 10 g/kg excelled against neck blast. Soil application of VAM @ 2 kg/ha proved to be most effective against finger blast (76.08 % reduction) and also yielded the highest fodder (7750.17 kg/ha) and improved growth. These findings provide a foundation for developing integrated blast management strategies in finger millet.

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Unraveling Mating Compatibility of *Pyricularia* spp., Infecting Rice and Millets

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RICE and millets are vital cereals, serving mainly as staple food crops and millets gaining importance as 'nutri-cereals', offering superior nutrition and climate-resilient benefits. Nevertheless, their productivity is severely threatened by blast disease caused by *Pyricularia* spp., heterothallic ascomycetes with the teleomorph *Magnaporthe*, resulting in major yield losses. In the present study, thirty blast isolates collected from Uttarakhand, Rajasthan, Tamil Nadu, Andhra Pradesh and Karnataka, were isolated using the spore drop technique. Morphological and molecular characterization identified as *Pyricularia* spp. The isolates were further validated and identified as *Pyricularia* spp. by comparison with original descriptions and BLAST analysis of the ITS gene. Potato dextrose agar supported greatest mycelial growth for foxtail millet (78.33 mm) and rice isolates (74.00 mm), whereas oat meal agar recorded highest growth for finger millet (83.66 mm) and pearl millet (43.33 mm) isolates, while rice straw extract agar was found effective for inducing sporulation. Mating type analysis using MAT gene-specific primers revealed that twelve isolates belonged to *MAT1-1* (male), fourteen to *MAT1-2* (female), and four exhibited both *MAT1-1* and *MAT1-2* (hermaphroditic) characteristics. All three mating types *viz*., male, female and hermaphrodite were observed in foxtail, finger and pearl millet isolates, whereas in rice only male and female mating types were present and female isolates slightly dominated across the population examined. Mating compatibility analysis was conducted among all isolates by making random pairings of male, female and hermaphrodite types in all possible combinations to induce perithecia formation at the points of contact. Despite multiple mating compatibility tests, no perithecia were formed, likely due to genetic variability or incompatibility among isolates. These findings highlight the reproductive diversity and polymorphic nature of *Pyricularia* spp., which have significant implications for understanding pathogen biology and developing effective blast management strategies.

Influence of Hydroponic Systems and Soilless Substrate on Growth, Yield and Quality of Strawberry (*Fragaria* × *Ananassa*)

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AN experiment was conducted at the Soilless Demonstration Unit, NSP, University of Agricultural Sciences, GKVK, Bengaluru to evaluate the impact of different soilless substrates and hydroponic systems on the growth, yield and quality of strawberry (cv. Sweet Sensation). The soilless substrates used for open system (Dutch Bucket system) of hydroponics were cocopeat, perlite and vermicompost whereas, for closed system of hydroponics (*i.e.,* Ebb and flow system and Nutrient Film Technique) cocopeat, perlite and vermiculite were used. The findings highlighted the importance of selecting suitable soilless media and nutrient regimes for optimal strawberry cultivation. In the Dutch bucket hydroponic system, the substrate mixture of T_5 (Cocopeat 70 % + Perlite 20 % + Vermicompost 10 %), proved most effective for enhancing growth, yield and quality. In the Ebb-flow system, the cocopeat and perlite (1:1) substrate performed best with respect to growth, yield and quality. On the other hand in the NFT system, the substrate with a higher proportion of perlite showed superior results compared to other treatments. Additionally, to examine the effect of different nutrient regime on growth, yield and quality of strawberry under Ebb and flow and Nutrient Film Technique, various concentrations of Hoagland solution (50, 80, 130 and 200%) were tried. A moderately high nutrient concentration (Hoagland solution at 130 %) yielded the best growth, yield and quality outcomes for strawberries grown in the Ebb and flow and Nutrient Film Technique hydroponic systems.

Evaluation of Jackfruit (*Artocarpus heterophyllus* Lam.) Genotypes for Vegetable Purpose through Morphological and Molecular Markers

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The present study aimed to identify elite jackfruit (*Artocarpus heterophyllus* Lam.) genotypes suitable for vegetable purpose from existing germplasm in and around Bengaluru during 2020-2022. A total of 54 genotypes were characterised based on morphological, biochemical, organoleptic and molecular characteristics of which 20 genotypes were selected for detailed study. Considerable variations were observed in morphological traits such as tree crown, leaf and fruit morphology. The dominant traits included broadly pyramidal crown, spreading growth habit, elliptic leaves with acuminate apex and green, ellipsoid fruits with spiny-rind. GTC-3 exhibited superior fruit weight (1.754 kg and 2.341 kg) and KV-1 recorded maximum edible portion recovery per cent (71.9 and 75.46 %) at six and eight-weeks fruit maturity, respectively. Biochemical characterization revealed minimal variations among genotypes. The higher ascorbic acid and lower phenol content was noticed in Byrachandra. Higher crude fibre content observed in KV-1 and NSP-1 with maximum potassium levels. Organoleptic evaluation ranked Byrachandra as superior with higher score for colour, aroma, taste and overall acceptability, followed by KV-1, HV-2 and GTC-1. Genetic variability and correlation studies indicated high heritability and significant positive correlations between mesocarp weight with fruit weight, fruit width and mesocarp thickness, indicating potential traits for vegetable purpose. Molecular diversity analysis using 20 SSR markers identified clear clustering and polymorphism which supported genetic differentiation. Overall, the genotypes KV-1, HV-2, GTC-3 and HV-1 were considered as promising vegetable types. Meanwhile, popular table purpose varieties *viz.,* Lalbagh Madhura, Byrachandra and Swarna showed potential to use for vegetable purpose at tender stage.

Efficacy of Bio-Stimulants on Growth, Yield and Quality of Okra (*Abelmoschus esculentus* (L.) Moench)

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This study examines the impact of bio-stimulants in enhancing plant growth, yield and quality parameters in okra (*Abelmoschus esculentus* (L.) Moench). The experiment was conducted during the late *rabi* season of 2023 at the Department of Horticulture, GKVK, Bengaluru to evaluate foliar application of Moringa Leaf Extract (MLE) and Seaweed Extract (*Ascophyllum nodosum*) (SWEA), both individually and in combination, across various concentrations. The results demonstrated that the combined application of MLE @ 35 ml/L + SWEA @ 4 ml/L was the most effective, leading to significant improvements in multiple growth parameters such as plant height (76.08 cm), leaf area (137.04 cm^2), fresh weight (113.17 g) and dry weight (72.42 g) of plant at 60 DAS. Additionally, significantly improved physiological and flowering traits, including earlier first flowering (32.8 days) and 50 per cent flowering (35.9 days) were recorded under this treatment. Yield attributes also showed remarkable improvement, with the highest pod length (17.37 cm), pod weight (14.06 g) and pod yield per plant (265.72 g), representing 40.3 per cent increase over the control. Seed yield attributes, such as the number of seeds per pod (38.80) and seed yield per plot (0.89 kg) and chlorophyll content (52.21 SPAD at 60 days) was also improved. The highest gross return (Rs.590,479.2) and net return (Rs.349,489.2) were achieved, resulting in a cost-benefit ratio of 1:2.45. The findings suggest bio-stimulants as promising alternatives to chemical fertilizers for improving crop quality and resilience under diverse growing conditions.

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Technical Feasibility and Economic Viability of Zero Budget Natural Farming - A Study in North Coastal Region of Andhra Pradesh

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NATURAL farming relies on natural processes and local resources and eliminate the need for costly external inputs. The present study in North coastal region of Andhra Pradesh used data from 40 farmers each practicing natural farming and conventional farming in each of the three major districts *viz.,* Vizianagaram, Parvathipuram Manyam and Alluri Seetha Ramaraju practicing natural farming. Data were analysed using logistic regression and Multinomial logistic regression, Bisaliah's decomposition model, Contingent valuation, other suitable analytical tools. Results of the study indicated that adoption of natural farming was higher among younger, educated and small farmers. The estimates of logistic regression revealed that education (0.2235), livestock ownership (2.3047), higher returns (0.0002), and health benefits (1.4604) were the key determinants for adopting natural farming. Natural farming reduced cultivation costs by 15 per cent, 14 per cent and two per cent for paddy, cotton and ragi, respectively, with relatively higher net returns than their conventional farming practicing counterparts. Carbon emissions were consistently lower from crops under natural farming compared to conventional practices. Major challenges in natural farming included intensive labour requirement, cumber some input preparation, low initial yields, limited marketing and consumer awareness. Value chain analysis revealed that farmers fetched premium prices for naturally grown paddy and ragi. The consumer preference analysis for naturally grown produce revealed that health, dietary inclusion and taste as major reasons for their choice but were unhappy with irregular availability and lack of certification. Educational status and income significantly influenced willingness to pay a premium for natural products. Study advocates enhanced support and policy interventions for promotion of natural farming through suitable certification procedures for better price realization and provision of incentives proportional to the extent of emissions reduction.

Modelling for Optimum Resource Allocation on Farms to Develop A Customized Crop Advisory -A Pilot Study in Chikkaballapur District, Karnataka

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AGRICULTURE is vital for sustainable India's livelihood. However, farmers face challenges in achieving high yields and good market prices. This study focuses on the Chikkaballapur district in Karnataka. Chikkaballapur has unique characteristics and profitability offers an opportunity to address these challenges and improve the overall agricultural sector. The basic linear programming (LP), MOTAD and goal programming (GP) techniques were used to obtain the optimum crop plan for the farms that optimize yield, returns and costs. A model is formulated for the 2.5 acres of farm for two seasons (*kharif* and *rabi*) with 15 crops were considered (9 *kharif* – maize, ragi, groundnut, horsegram, tomato, paddy, pigeonpea and fieldbean and 6 *rabi* crops – maize, cowpea, ragi, potato, tomato, paddy) and 3 dairy breeds (local cow, cross breed and buffalo). The vital parameters of the study *viz*., cost of cultivation of major crops, area, production and productivity and carbon emissions data were extracted from the CACP, DES and Cool farm tool, respectively. The goals considered for model were to maximizing gross returns, minimizing costs and minimizing carbon emissions. Two feasible solutions were obtained from LP, MOTAD, and GP models with LP and GP solutions being the most feasible for the region. The returns obtained by LP and GP plans were Rs.407078 and Rs.386403 per hectare, respectively. LP model was validated with primary data (small, medium and large farm lands) and model found most optimum plan with minimum allocation. The net returns obtained by optimal plans were higher with per cent change of 139.77, 87.26 and 78.81 per cent for small, medium and large farmers, respectively. The Euclidean distance between plans was found higher in *rabi* season (1.73) indicating optimal plans were farther from the existing crop plans. The model suggested optimized, sustainable and profit earning crop plans.

Performance of Canal Irrigation System and its Impact on Farmers' Livelihood in Narayanpur Left Bank Canal Command Area of Upper Krishna Project - An Economic Analysis

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CANAL irrigation has been a cornerstone of Indian agriculture, yet inefficiencies like inequitable water distribution, losses and mismanagement have reduced its effectiveness. The Narayanpur Left Bank Canal (NLBC) under the Upper Krishna Project in Karnataka faces these challenges with head-reach farmers enjoyed greater access to water compared to other reaches. To address this, automation in canal system was introduced for timely, efficient and equitable water distribution. This study analysed the water budget, performance of the canal system, economics of major crops and the impact of automation on farmers' livelihoods across the reaches. The research utilized secondary data and primary data, applying performance indicators, CAGR, Cuddy-Della Valle Index, livelihood indices, Cohen's d statistics and enterprise economics. The Results revealed that NLBC supplied 26.21 MHaCm of canal water and 5.06 MHaCm rainfall annually, meeting only 72 per cent of demand and leaving a deficit of 4.91 MHaCm (15.7%). Water shortages were most acute in *rabi*/summer (-23.73%). Irrigated area expanded significantly (CAGR: 8.53%), with all branch canals surpassing targets. However, waterlogging (23,815 ha), salinity and alkalinity remain major concerns. Automation improved equity and effectiveness across the reaches. Livelihood security indices rose by 24.24 per cent in the tail end, 13.89 per cent in the mid and 10.26 per cent in the head reach, with a strong intervention effect (Cohen's d=1.13). Incomes increased substantially, especially in the tail reach farmers (+25.84%). Improvements were also noted in food consumption, health access, housing, education, credit and savings. Crop enterprise analysis confirmed profitability with sugarcane crop being the most profitable (Rs.1.53 per rupee), followed by cotton (1.24), paddy (1.23) and groundnut (1.20) crop. The study concluded that automation helped achieve better water-use efficiency, equity and livelihood. It recommends scaling such reforms to other command areas, coupled with farmer capacity-building and public-private partnerships for sustainable irrigation management.

A Feasibility Assessment of Women Self-Help Groups to Promote Climate-Smart Agriculture (CSA) Technologies in Bihar: An Economic Analysis

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WOMEN'S participation in agriculture has expanded in Bihar, yet gender disparities persist due to unequal access to land, credit, technology and extension services. Climate change has further intensified these vulnerabilities with rural women increasingly responsible for farm management amid male outmigration. This study examines the role of Self-Help Groups (SHGs) in promoting women's participation in Climate-Smart Agriculture (CSA) technologies across rural Bihar. Using data from 1,200 farm households, it further analysed the determinants of SHG participation, women's empowerment in agriculture, awareness and adoption of CSA practices and their willingness to adopt and pay for digital extension services related to CSA technologies. Random Forest and Probit analyses identified caste, landholding, age, education, and wealth as key determinants of participation. Most SHG members were from marginalized castes (74 % OSMC; 18 % SC/ST) and smaller landholders (0.97 ha vs 1.50 ha). SHG women showed greater autonomy in seed sourcing (10.3 % vs 1.7 %), crop variety choice (9.3 % vs 3.2 %), and marketing (8.9 % vs 1.9 %) with higher empowerment scores (A-WEAI = 0.63 vs 0.47). Despite active involvement, empowerment gains among SC/ST women remained limited, suggesting intra-group inequalities. Awareness and adoption of CSA practices were significantly higher among SHG households; About 22.90 per cent were high adopters compared to 16.40 per cent of non-members. Exposure to climate shocks, landholding, and livestock ownership increased adoption intensity, while migration and tenant status reduced it. Despite comparable smartphone ownership, only 8.80 per cent of SHG women used phones for agriculture. Still, 60 per cent were willing to download CSA advisory apps compared to 47 per cent of non-members. SHG membership and smartphone access increased willingness to pay (WTP) for digital CSA seed delivery by Rs.138 and Rs.87, respectively. Overall, SHGs effectively enhanced women's empowerment, CSA adoption and digital readiness. Targeted, caste-sensitive interventions integrating digital literacy and labour-saving innovations can further strengthen SHGs as inclusive vehicles for climate-resilient agriculture in Bihar.

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An Analysis of Agri-Business Performance and Market Linkages of Selected Farmers' Producer Organizations in Shivamogga District of Karnataka

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This study explores the role of Farmers' Producer Organizations (FPOs) in improving agribusiness performance, market linkages and farmers' incomes in Shivamogga district of Karnataka. The study focused on two FPOs *viz.,* Malnad Horticulture Farmer Producer Company Limited (MHFPCL) and Sharavaathi Horticulture Farmer Producer Company Limited (SHFPCL). These FPOs play a crucial role in supplying agricultural inputs particularly pesticides which comprises significant share of cultivation expense. FPOs offer custom hiring services for farmers, provides tractor on rental basis to members at Rs.950/ hour in MHFPCL at Rs.150 less than the market price while SHFPCL's rental rate was Rs.900/hour and members saved Rs.200. These services are particularly beneficial for smallholder farmers who cannot afford to purchase such machinery. MHFPCL's current ratio increased from 3.76 in 2020 to 15.50 in 2023 and respective values for SHFPCL portion of FPC's were 28.91 and to 47.78 indicating stronger liquidity. However, MHFPCL's reliance on debt grew from 0.29 to 0.90 whereas SHFPCL remained stable at 0.52 during the study period. Among the backward linkages for MHFPCL the supply of seeds was the most effective followed by pesticides and fertilizers while farm machinery ranks lowest. In forward linkages marketing of agricultural produce was the most critical followed by storage and transportation facilities. In SHFPCL the supply of seeds was the most effectively managed service followed by fertilizers and pesticides while farm machinery ranks lowest. In forward linkages marketing of agricultural produce was the most effective followed by transportation facilities. The grading of produce ranks lowest suggesting the need for improvement. In arecanut, maize, paddy and ginger cultivation, members earned better returns per rupee spent with ratios of 2.30, 1.68, 1.23 and 2.88, respectively. The challenges faced by the FPOs were distant located markets, logistical issues such as transportation shortages and meeting desired quality standards.

An Economic Analysis of Production and Price Volatility of *Rabi* Sorghum in Belagavi District, Karnataka

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The present investigation was undertaken in Belagavi district of Karnataka, as Belagavi district plays vital role in the state's production of sorghum. The research examined the key aspects such as the socio-economic characteristics of farmers, costs and returns associated with sorghum production and also it study the resource-use efficiency, marketing channels and price volatility of *rabi* sorghum. The results of the study revealed that the average age of farmers was 49 years with 77.50 per cent of farmers were relying on agriculture as their primary occupation. The average land holding of sorghum cultivation was 2.15 acres. The cost analysis indicated that the total cost of cultivation of sorghum was Rs.17,607 per acre with variable costs accounting for 62.10 per cent and fixed costs 37.90 per cent. The average yield realised was 4.10 quintals per acre, generating the net returns of Rs.108.21 per acre. The return per rupee of expenditure was 1.01 indicating the crop's economic viability. The Cobb-Douglas production function analysis highlighted that fertilizer and machine labour were the significant factors in improving the productivity. The study also evaluated marketing efficiency, showing that direct marketing from farmers to consumer gave the highest producer's share in consumer rupee (99.50 %). The price forecasting using SARIMA model and volatility analysis through GARCH model highlighted seasonal price fluctuations. The findings provided the insights into optimizing the resource allocation, improving marketing strategies and stabilizing the prices of *rabi* sorghum in the region.

Impact of e-NAM on Marketing of Pulses - A Case in Kalaburagi District of Karnataka

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THE present study was undertaken in Kalaburagi district of Karnataka to analyse the extent of adoption of e-NAM in marketing of pulses (redgram and bengalgram), impact of e-NAM on price discovery and arrivals, trading benefits of e-NAM versus conventional marketing and constraints faced by different stakeholders in e-NAM platform. The primary data was collected from 170 samples, 140 farmers, which includes 70 redgram growing farmers and 70 bengalgram growing farmers , 10 traders, 10 commission agents and 10 market officials. The data were analysed using tabular analysis, growth rate analysis, instability analysis, descriptive statistics analysis, logit regression analysis and Garretts ranking. The results revealed that the growth rate (2012-13 to 2022-23) of redgram recorded 6.16 per cent in production and 1.21 per cent in productivity, while bengalgram showed a negative growth rate of -15.18 per cent in production. Price realization of redgram increased significantly under e-NAM, reaching Rs.11,502/q compared to Rs.10,378/q in conventional markets and for bengalgram reaching Rs.6,093.13/q compared to Rs.5,525.41/q in conventional markets. Similarly, net gain was higher of Rs.1366.84/q for redgram and Rs.718.93/q for bengalgram under e-NAM compared to conventional markets. Transaction time under e-NAM was reduced to 1 day compared to 2.87 days in traditional systems. Constraints such as farmer illiteracy, lack of proper dissemination of information, existence of cartels among traders and limited infrastructure hindered effective participation. Findings of the study recommends need for improved awareness, helpdesk, electronic display boards, digital literacy and infrastructure to enhance farmer participation and efficiency in e-NAM platforms.

An Analysis on Perception of Beekeepers towards Beekeeping in Selected Agro-Climatic Zones of Karnataka

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THE present study was carried out during 2022-23 to analyse the perception of beekeepers towards beekeeping in selected agro-climatic zones of Karnataka. A total of 180 beekeepers were randomly selected from Coastal (Dakshina Kannada district) (60 Nos.), Hilly (Kodagu district) (60 Nos.) and Southern dry (Chamarajanagar district) (60 Nos.) zones of Karnataka. Data was collected using a pre-tested interview schedule. Nearly three-fourth of the beekeepers (74.45%) had good to better overall perception towards beekeeping, whereas, 71.67 per cent of the beekeepers had medium to high level of overall knowledge regarding scientific beekeeping practices and a majority of the beekeepers (71.67%) were belonging to medium to high overall adoption category of scientific beekeeping practices. Education, farming experience, number of bee hives, experience in beekeeping, innovativeness, achievement motivation, scientific orientation, risk orientation, cosmopoliteness, decision-making ability, market orientation, economic motivation, mass media exposure, training on beekeeping, extension agency contact, extension participation of beekeepers had significant to highly significant relationship with their perception, knowledge and adoption of scientific beekeeping practices in all the three agro-climatic zones. Lack of knowledge on colony management, dearth period management and management of colonies during monsoon season, lack of knowledge on management of wax moth pest and Thai sac brood viral disease, lack of subsidy for beekeeping equipments, non-availability of credit facilities for rearing honey bees, low price for honey/ bee products and delay in payment by co-operative societies were the problems faced by the beekeepers in the three agro-climatic zones of Karnataka.

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Impact of Front Line Demonstrations (FLDs) of Krishi Vigyan Kendras in Tumakuru district of Karnataka: A Critical Analysis

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The present study was conducted to evaluate the attitude and impact of frontline demonstrations (FLDs) implemented by two Krishi Vigyan Kendras (KVKs) in Tumakuru district. The assessment was based on key indicators, including knowledge acquisition, technology adoption, yield & economic improvement and social recognition. A list of FLD partner farmers cultivating finger millet, red gram, groundnut and paddy was obtained from Tiptur (UASB) and Hirehalli (ICAR-IIHR) KVKs. From this list, 40 partner farmers from each crop were selected, resulting in a total sample size of 160. Data were collected using pre-tested interview schedule and a standardized scale was employed to measure farmers attitudes towards FLDs. The findings revealed that 42.50 per cent of respondents exhibited a more favorable attitude towards FLDs, whereas, 32.50 and 25.00 per cent of farmers having favorable and less favorable attitudes, respectively. Prior to the implementation of FLDs, two fifth (40.00 %) of the respondents belonged to the medium impact category. However, following the intervention, a notable shift was observed, with 76.87 per cent of respondents transitioning to the medium to high impact category. In the stepwise regression analysis, scientific orientation was initially the only factor included, explaining approximately 20.10 per cent of the variation in the attitude levels of partner farmers. Likewise, information-seeking behaviour was the sole factor influencing the impact of FLDs among partner farmers, accounting for about 75.80 per cent of the variation. The major challenges encountered in FLD implementation included shortage of labour and high wages, inadequate irrigation facilities, unremunerated & fluctuated market prices and exploitation by intermediaries. To enhance the effectiveness of FLDs, KVKs should prioritize the dissemination of timely weather information, the development of labour-saving technologies, capacity-building initiatives through training programmes and the provision of adequate irrigation provisions to facilitate the successful adoption of improved agricultural practices.

A Comparative Analysis of Perception and Satisfaction of Teachers and Students towards Offline and Online Classes in Selected State Agricultural Universities of Karnataka

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The present study was carried out during 2023-24 to analyse the perception and satisfaction of teachers and students towards offline and online classes in selected State Agricultural Universities of Karnataka. A sample of 90 teachers and 90 students each from University of Agricultural Sciences, Bangalore and University of Agricultural Sciences, Dharwad were selected for the study. Data was collected from the respondents using pre-tested interview schedule. The results revealed that a majority of the teachers (57.78 %) and students (54.44 %) of both the universities had better perception towards offline classes, further it was found that a greater proportion of teachers (52.78 %) and students (41.11 %) of both the universities had better perception towards online classes. The results also revealed that a vast majority of the teachers of UASB (83.34 %) and UASD (82.22 %) were feeling satisfied and more satisfied towards offline classes, respectively, besides more than three-fourth of the students of UASB (78.89 %) and UASD (75.55 %) were also feeling satisfied and more satisfied towards offline classes. Difficulty in managing large class sizes and longer schedules of classes are sometimes much boring to attend, were the major problems faced by teachers and students in offline classes, respectively. Internet/ Network related difficulties interrupting lessons frequently and difficulty in understanding complex concepts without in-person explanations and demonstrations were the major problems faced by teachers and students in online classes, respectively.

Problems and Prospects of Potato Growers in Hassan District of Karnataka

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THE study was purposively conducted in Hassan district to analyze the problems and prospects of potato growers during 2023-24. The primary data was collected from randomly selected 120 potato growers by personal interview method from Hassan and Arakalgud taluks. The *ex-post-facto* research design was adopted and the findings revealed that nearly two-fifth (39.17 %) of potato growers had medium overall performance followed by high (31.66 %) and low (29.17 %) categories. Specifically, 40.83 per cent of growers demonstrated medium knowledge performance and more than half (53.33 %) of them had medium adoption performance. Whereas, more than one-third of them belonged to medium economic performance (35.83 %), high social performance (39.17 %) and medium yield performance (44.17 %) categories. The average Benefit-Cost (B:C) ratio for potato growers was 1.61, while the average yield stood at 40.09 quintals per acre, with a yield gap of 33.18 per cent. The key variables such as education, annual income, farming experience, extension contact, information-seeking behavior and innovativeness had a significant association with performance of potato growers at 5 per cent level. The compound annual growth rate of area, production and productivity of potato crop was declined by -8.88 per cent, -8.95 per cent and -0.946 per cent, respectively during 2001-02 to 2023-24 in Hassan district. The major constraints experienced by growers included pest and disease outbreaks, high labor costs, low market prices, unavailability of quality seeds and inputs, and high production costs. The farmers expressed the development of cost-effective technologies for late blight management, availability of quality seed tubers, improved potato varieties and timely access to input and market information were important suggestions. These measures could enhance productivity and returns, addressing the challenges faced by potato growers in the region.

Analysis of ICT Enabled Learning Approaches among Agricultural Students in Karnataka

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INFORMATION and Communication Technology (ICT) services are transforming the global landscape. These technologies are seen as powerful tools to enhance educational services and economic efficiency. The study was conducted in three agricultural Universities of Karnataka *viz.,* UASB, UASD, KSNUAHS during 2023-2024 to analyse the knowledge, attitude and extent of utilization of ICT enabled learning approaches among agricultural students in Karnataka. A total of 120 students comprising 40 under graduate students from each Universities were selected for the study using simple random sampling. *Ex-post facto* research design was used. Agricultural Universities are equipped with ICT facilities such as digital library, wi-fi facility, smart classrooms, plagiarism software, OMR based evaluation, University websites, social media platforms and facilities like computers and photocopy machines. More than two fifth (44.16 %) of the students belonged to medium knowledge level category, followed by high (30.00 %) and low (25.83 %) knowledge levels. It was found that 35 per cent of the respondents had favourable attitude, followed by more favourable (34.17 %) and less favourable (30.83 %) attitude. It was also found that more than two fifth (42.50 %) of the students had medium level of extent of utilization, followed by high (33.33 %) and low (24.17 %) level of utilization. Variables such as ICT exposure, rural/urban background, academic achievement, innovative proneness, time spent on gadgets and mass media utilization were found to have significant association with knowledge, attitude and extent of utilization. Major problems expressed by students were 'Lower/unstable internet bandwidth' and 'Less training about how to use ICT'. Major suggestions were '24x7 wi-fi facilities should be made available to the students in colleges' and 'Periodical training programmes on application of ICT'. Hence, the state need to assist Universities to prioritize enhance ICT infrastructure, digital literacy and prepare students for a digitally driven world.

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Adoption of Production Technologies and Entrepreneurial Behaviour of Mango Growers in Eastern Dry Zone of Karnataka

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Mango cultivation is driving the development of a robust value chain across India, leveraging horticulture's potential for higher unit productivity and significant value addition. The study was conducted in Srinivasapura taluk of Kolar district and Chintamani taluk of Chikkabalapur districts of Karnataka during 2023-2024 to analyse adoption of production technologies and entrepreneurial behaviour of mango growers in Eastern Dry Zone Karnataka. The study sample consisted of 120 mango growers, with six villages selected from each taluk and ten growers, comprising of 5 big farmers and 5 small farmers chosen from each village. *Ex-post facto* research design was used. Exactly two fifth (40.00 %) of the mango growers had medium level of adoption, followed by low (33.33 %) and high (26. 67 %) level of adoption. Nealy two-fifth (37.50 %) of the farmers belonged to medium entrepreneurial behaviour category, followed by low (35.00 %) and high (27.50 %) categories of entrepreneurial behaviour. Variables such as education, annual income, extension contact, extension participation, mass media exposure, cosmopliteness, social participation and level of aspiration were found to have a significant relationship with the adoption of production technologies and entrepreneurial behaviour. Major technical constraint was, 'high susceptibility of disease and pest', economic constraint was, 'high cost of establishment of mango orchard', storage constraint was, 'high fluctuation in market prices' and processing and value addition constraint was, 'absence of value addition units in the area'. Major suggestions were to 'provide information on pest and disease management in mango orchards' and 'Government should establish mango processing units and storage structures in mango growing areas'.

Cognitive Level and Adoption Behavior of Exotic Vegetable Growers in Bengaluru Rural District of Karnataka

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The present study was conducted in Bengaluru Rural district of Karnataka in the year 2023-2024 to study the cognitive level and adoption behavior of exotic vegetable growers towards scientific farming technologies. Hoskote and Doddaballapura taluks were selected for the study. From each taluk, 25 farmers each growing broccoli and lettuce were selected for the study. Thus, making a total sample size of 100. The findings of the study revealed that more than one-third (36.00 %) of broccoli growers had medium cognitive level, followed by high (34.00 %) and low (30.00 %) about scientific crop production technologies. Nearly two-fifth (38.00 %) of the lettuce growers belonged to medium cognitive level category, followed by high (32.00 %) and low (30.00 %) categories. Thirty-eight per cent of broccoli growers had medium adoption, followed by high adoption (34.00%) and low adoption (28.00%). More than one-third (36.00 %) of lettuce growers had medium adoption, followed by low adoption (34.00 %) and high adoption (30.00 %) category. Majority of the exotic vegetable growers possess medium to high economic performance. The variables *viz.,* education, extension contact, extension participation, risk orientation, innovativeness and scientific orientation had positive and significant association with adoption level of exotic vegetable growers. The major constraints expressed by exotic vegetable growers were market price fluctuations, non-availability of timely skilled labour, perishability of produce and occurrence of pest and diseases. The major suggestions given by the exotic vegetable growers were encouraging contract farming practices, availability of quality and timely inputs, crop rotation practices and government support policies.

A Study on Utilizaton of Warehouse Services in Karanataka - A Business Analysis

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Warehousing is essential for trade, commerce and agricultural goods distribution, helping farmers avoid distress sales and price fluctuations during peak seasons. By a scheduled commercial bank from key benefit for stakeholders like farmers and traders is financial assistance from pledging Negotiable Warehouse Receipts (NWRs) received against storage from the Karnataka State Warehousing Corporation (KSWC). This research aimed to examine storage utilization and services in Karnataka. So, goal of the study was to analyse utilization patterns, costs, returns, demand-supply gaps and challenges in warehousing services. The data were gathered through personal interviews with 70 stakeholders and KSWC staffs across seven regions, supplemented by secondary data from KSWC reports from 2014 to 2023. Findings revealed that agencies dominated storage (78.52 %), followed by FCI (14.79 %), farmers (5.46 %) and traders (5.28 %) with 77,121.78 MTs and 74,617.53 MTs stored, respectively. KSWC total owned storage capacity for 2023-24 was 1,413,473 MTs, with warehouse utilization rising to 1,470,696.75 MTs in 2024. Cost and returns showed favorable benefit-cost (B:C) ratios for crops like maize (2.58) and Bengal gram (2.59) with storage duration during *rabi* and *kharif* seasons (< 6 months & 6 months to 1 years), respectively. The benefit accrued to farmers storing of tur was 54.55 per cent and 6.82 per cent for Bengal gram with 12 per cent interest opportunity cost. The demand-supply gap in storage capacity decreased from 75.28 per cent to 24.23 per cent from 2014 to 2024, though Bengaluru Urban (193.33 %) and Bengaluru Rural (169.23 %) faced the highest gaps. KSWC warehouse efficiency was affected by damaged structure, low stock receipt, labour shortages and procedural changes. With 41,876 MTs of warehouse capacity needing repair, the study suggested constructing new warehouses and repairing existing ones to benefit stakeholders.

Strategies for Enhancing Competitiveness of Goat Rearing in Sri Sathya Sai District of Andhra Pradesh

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The study examines the socio-economic characteristics, costs, returns, marketing practices and market structure of goat-rearing farmers in Sri Sathya Sai district, Andhra Pradesh. A sample of 90 farmers, classified into small (<40 goats), medium (41-60 goats) and large (>60 goats) herd sizes was analyzed. Middle-aged individuals (46-60 years) managed most large herds 58.62 per cent, while illiteracy was prevalent among all categories, affecting 43.33 per cent overall and 55.17 per cent among large herd owners. Goat rearing served as the primary occupation for 93.33 per cent of farmers, with nuclear families forming the majority (81.11%). Larger herds demonstrated higher economic returns, with net incomes ranging from Rs.71,677 for small herds to Rs.1,88,689 for large herds, alongside an improvement in the benefit-cost ratio from 2.55 to 2.76. Feed was the largest cost component, accounting for 34 per cent of total expenses, followed by veterinary care, which averaged Rs.24,509 annually. Marketing practices were largely traditional with 60.15 per cent of farmers preferring weekly village markets (shandies), followed by middlemen (49.71%) and district markets (47.50%). Key factors influencing prices included gender, weight and market demand with male goats and heavier animals fetching higher rates. Farmers faced challenges such as finding reliable buyers (59.56%), competition during low-demand periods (58.84%) and difficulty securing fair prices (50.95%). Market structure analysis using the Herfindahl-Hirschman Index (HHI) revealed competitive conditions with low buyer concentration, enabling farmers to negotiate fairer prices. The findings show how important goat farming is to rural livelihoods, emphasizing how herd size and effective marketing impact success. At the same time, it highlights the everyday challenges farmers face in running and growing their operations.

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Strengthening the Export Potential of Acid Lime from India : An Empirical Study

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STUDY was conducted in Vijayapura district of Karnataka, assessed farmers awareness of export quality standards, challenges and opportunities in acid lime cultivation and trade. Data was collected from 60 farmers through structured interviews and secondary sources such as APEDA, ITC trade maps and horticulture department reports were analyzed. Analytical tools like Nominal Protection Coefficient (NPC), Herfindahl-Hirschman Index (HHI), Compound Annual Growth Rate (CAGR) and Garrett's ranking technique were employed for analysis. The district had 12,788 ha under acid lime cultivation with an average productivity of 25.84 t ha^{-1}, which was significantly higher than the national average of 11.80 t ha^{-1}. Middle-aged farmers (63.33%) dominated lime farming, while small and marginal farmers accounted for (26.67%) of the total. Bore wells served as the primary irrigation source (95%), supplemented by canals (46.66%). Export competitiveness, as measured by the Nominal Protection Coefficient (NPC) of (0.62), indicates a moderate efficiency level in the acid lime export sector. Additionally, the Herfindahl-Hirschman Index (HHI) value of (0.423) demonstrates a highly concentrated export market. Markov Chain analysis showed that UAE market holds a significant (72.6%) share in Indian lime exports. Farmers faced significant challenges such as water scarcity, high labour costs, price fluctuations and inadequate awareness of export standards. Despite widespread adoption of innovative packaging, post-harvest handling and quality compliance remained limited. The study highlighted that acid lime exports are concentrated in Nepal and UAE, posing risks expanding to markets like Kuwait and Qatar can enhance stability. Addressing quality standard gaps through training on certifications and parameters can boost exports to high-value markets of European countries.

Effectiveness of Digital Marketing Strategies on Fast Food Consumption in Bengaluru

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THE study explored the impact of digital marketing strategies on consumer behaviour in the fast-food industry. It examined the effectiveness of digital marketing channels such as social media, e-mail marketing, search engine advertisements and influencer marketing in shaping consumer preferences and purchasing decisions. Primary data was collected from 120 respondents, capturing demographic factors like age, education, income and dietary preferences. Analytical tools such as logistic regression and decision tree analysis were employed to identify significant factors influencing consumer behaviour and to illustrate the pathways through which digital marketing strategies impacted fast-food consumption patterns. The findings revealed that 90.83 per cent of fast food consumers were young, 56.67 per cent were graduates and 38.33 per cent earned Rs.30,000 – Rs.40,000 per month. Female fast food consumers (56.66 %) showed a slightly higher preference for fast food compared to males (43.33 %) and 60 per cent of respondents were non-vegetarians. Majority of the consumers (85%) have strong preference for fast food with 47.5 per cent consuming it at least once in a week. Burgers (91.66%), pizza (88.33%) and fried chicken (85.83 %) were the most popular items. Social media influenced 77.5 per cent of respondents with online reviews and discounts emerging as key motivators. Videos (84.16 %) and photos (74.16%) were the most engaging content types, followed by blogs (71.66 %). Influencer recommendations impacted 66.67 per cent, while search engine advertisements influenced 72.5 per cent. Domino's (65.8 %) and KFC (60.8 %) were the most recognized brands, with Domino's social media campaigns significantly influencing 70 per cent of respondents. The study concluded that leveraging visual appeal, convenience and financial incentives through digital tools is pivotal in shaping consumer behaviour, optimizing marketing strategies and building brand loyalty in the competitive fast-food industry.

Competitiveness of Bengalgram in SPSR Nellore District of Andhra Pradesh

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INDIA contributed (70%) of world's bengalgram production with 13.75 m.t. annually from 10.91 m.ha. and productivity of 12.60 qt ha^{-1} in the year 2022. Andhra Pradesh occupied 6.89 per cent of India's area under bengalgram and SPSR Nellore was the major bengalgram producing district during the same year. This study was carried out to understand the growth and instability in area, production and productivity of bengalgram, to analyze the technical efficiency and allocative efficiency in its production and to identify major constraints faced by farmers. The study used both primary data collected from farmers and secondary data on the area, production and productivity of bengalgram from the year 2014-2024. Data collected was analyzed using CAGR, stochastic frontier production function and Garrett's ranking. The results indicated significant growth in the area (5.93%), production (4.89%) and productivity (-0.99%) at five per cent significance and instability was observed more in production (43.50%) compared to area (37.23%) and productivity (10.62%). The study also indicated 80 per cent of farmers were very high (>90%) and 13.33 per cent of farmers were having high (80–90%) technical efficiency, indicating most of them operated near their potential. In bengalgram production inputs like fertilizer (0.04), plant protection (-0.31) and seed (0.29) were overutilized while labour (2.32), machine (2.11) and manures (1.17) were underutilized. Inadequate rainfall (70.75) and pest and disease infestation (59.93) were the major production constraints, while inadequate storage facilities (69.78) and defective and faulty weighing (62.06) were the major marketing constraints faced by farmers. SPSR Nellore district being a major bengalgram growing area, the study recommended, enhancing infrastructure facilities can significantly reduce post-harvest losses. Additionally, the study recommended strengthening bengalgram farmers through Farmer Producer Organizations (FPOs) and cooperatives to empower collective buying of inputs and collective marketing of produce.

Profitability Analysis of Tobacco Production and its Value-Added Products in Bihar

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THE present study on profitability analysis of tobacco cultivation, marketing and constraints analysis for value added products of tobacco was carried out in Vaishali district of Bihar. The required primary data related to 2023-24 season were collected from 60 tobacco growers, 30 traders and 30 consumers of the value added products through personal interviews. Results indicated that 63.33 per cent of farmers rely on agriculture as their primary income source, followed by cattle and poultry farming. Respondents predominantly included middle-aged males with varying educational qualifications, with SSLC qualification being the most common. The dominance of *kharif* crops was seen in area utilization, while *rabi* crops exhibited higher productivity. The hingla variety is leading variety of tobacco cultivated (30%), suited for cigarettes, followed by bidi, chewing tobacco and hookah varieties. The total cost of cultivation dominated by labor cost with favorable returns of Rs.1.98 per rupee spent. The production of value-added products like bidis, chewing tobacco and cigarettes were found profitable with the highest consumer preference for chewing tobacco. Among the various Marketing channels, Channel-I was the most lucrative quantity traded, while Channel-III was most preferred (36.67%). Farmers need to be trained on cost effective production practices better resource use and post-harvest handling for quality production for better price realization and profits in domestic and international markets. By synthesizing these factors, the study sheds light on the profitability landscape of tobacco cultivation in Bihar, offering valuable information for farmers, policymakers and stakeholders involved in agricultural decision making.

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Economics of Production and Marketing of Farm Enterprises in Kolar District

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THE study focused farm enterprises in Kolar district with the objective of analysing profitability of different farming systems [(C+Sh), (C+D), (C+D+H), (C+D+S)], employment and income generation, marketing channels of different farm products and constraints involved in taking up farm enterprises. The primary data was collected from 30 growers and 17 market intermediaries. Capital budgeting technique, Descriptive statistics, Acharya's method and Garrett's ranking techniques were used to analyse the data. The returns per rupee of expenditure was highest in C+D+H (2.21) followed by C+Sh (1.91), C+D+S (1.74) and C+D (1.44). Net income of farm households was highest in C+D+S (Rs.3,76,453) followed by C+D+H (Rs.2,41,089), C+D (Rs.2,06,906) and C+Sh (Rs.1,09,755). Labour employment generation was found to be highest in C+D+H with 1,580 labour days per annum. The efficient marketing channel in C+Sh farming system was Channel-I (Farmer - APMC) in the case of crops and Channel-II (Farmer - Butcher - Consumer) in the case of sheep. The efficient marketing channel for dairy was Channel-I (Farmer - Consumer) in the case of all farming systems. The efficient marketing channel in C+D+S was Channel-I (Farmer - Wholesaler – Consumer [Reeler]) for sericulture. The efficient marketing channel in the case of C+D+H farming system was Channel-I (Producer - Consumer) for horticulture crops. Shortage of labour and low price for the produce were the major production and marketing constraints faced by the farmers. Farmer Producer Organizations (FPOs) and Co-operative Societies serve as an important platform for farmers to get high prices for their produce.

Studies on Crop Weather Modelling in Groundnut (*Arachis hypogaea* L.)

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STUDIES on crop weather modelling in groundnut (*Arachis hypogaea* L.) was carried out during *kharif* 2023 and 2024 at the AICRP on Agro-meteorology field unit, Zonal Agricultural Research Station, UAS, GKVK, Bengaluru under rainfed conditions. The experiment was laid out in a factorial RCBD with three groundnut varieties (KCG-6, K-1812 and K-6 and two dates of sowing (June 2nd fortnight and July 1st fortnight, replicated four times. Results showed that the July 1st fortnight sowing recorded significantly higher pod yield (2799 kg ha^{-1}), harvest index (0.30), No. of pods (27.96), pod weight (35.81 g), plant height (49.35 cm) and number of branches per plant (10.30), compared to the June 2nd fortnight sowing. Among genotypes, K-1812 exhibited superior performance with significantly higher pod yield (3603 kg ha^{-1}), haulm yield (15077 kg ha^{-1}), No. of pods (36.88), pod weight (41.28 g), total dry matter production (92.38 g), no. of branches (11.53), leaf area (287.94 cm^2) and LAI (0.96) at harvest. The interaction revealed that K-1812 sown in June 2nd fortnight achieved the higher pod yield (3510 kg ha^{-1}). Higher GDD, PTU, HETU, HYTU, HUE, PTUE, HyTUE and RUE were noted in July 1st fortnight sowing. These findings emphasize the importance of selecting appropriate sowing windows and high-yielding cultivars to optimize groundnut productivity under varying climatic conditions. The DSSAT model was more accurate in simulating groundnut yield during 2024 than 2023, with K-1812 showing the most consistent performance and KCG-6 exhibiting better model fit (D = 0.804). Overall, the model performed better for June 2nd fortnight sowing with higher agreement (D = 0.986).

Studies on Utilization of Marigold Flower Biowaste on Growth and Yield of Maize (*Zea mays* L.)

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The study was carried out with laboratory and field experiments conducted during Summer 2023 and 2024 at ZARS, UAS, GKVK, Bengaluru and conclusion were drawn. Marigold flower liquid biowaste (MFLB) and enriched marigold flower liquid biowaste (EMLB) exhibited neutral pH, EC ranged from 0.29 to 0.31 dS m^{-1} and BOD, COD, dissolved oxygen below permissible limits. Nitrogen was higher in MFLB (0.18%) and EMLB (0.20%) with EMLB recording higher macronutrients, bacterial (294.29%) and fungal populations (222.08%), dominated by Proteobacteria (51.09%) and Gammaproteobacteria (47.69%). GC-MS analysis revealed EMLB rich in esters, alcohols and carboxylic acids, while MFLB predominated in alcohols, phenols, carboxylic acids and alkenes. The treatments includes three factors *viz.,* MFLB application, dosage of application (75:25, 50:50, 25:75 and 100%) and time of application (Basal and tasselling stage). Among, 16 treatments application of 75 per cent N through RDN + 25 per cent N through EMLB (basal + tasseling) recorded significanlty higher grain (10075 kg ha^{-1}) and stover (16479 kg ha^{-1}) yield, with significantly higher plant height (223.28 cm) and leaf area (5833.83 cm^2). Similarly, application of 50 per cent N through RDN + 50 per cent N through MFLB resulted in higher gross returns (Rs.1,80,738 ha^{-1}), net returns (Rs.1,37,228 ha^{-1}), and B:C ratio (4.184). Among, marigold solid biowaste composts, C_4 compost (Marigold flower solid biowaste + 5% cow dung + rock phosphate + waste decomposer + EMLB), recorded higher nutrients N (4.49%), P (0.98%) and K (3.23%) with domination of Proteobacteria (36.64%) and Actinobacteria (29.09%). Field application of C_4 compost @ 10 t ha^{-1} + RDF significantly recorded higher plant height (230.99 cm), leaf area (7813.42 cm^2) and dry matter accumulation (265.99 g), higher grain yield (9315 kg ha^{-1}), higher stover yield (18291 kg ha^{-1}) and higher gross returns (Rs.1,67,670), net returns (1,31,643 ha^{-1}), and B:C (4.65).

Response of Fodder Maize (*Zea mays* L.) to the Pollutants of Treated Wastewater Irrigation

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A field experiment entitled 'Response of fodder maize (*Zea mays* L.) to pollutants of treated wastewater irrigation' was conducted during the summer of 2021 and 2022 at the Agro-forestry Unit (H-7 Block), Zonal Agricultural Research Station, Gandhi Krishi Vignyana Kendra (GKVK), University of Agricultural Sciences, Bangalore. The experiment consisted of three irrigation sources *viz.,* borewell water, secondary treated waste water and tertiary treated waste water and four graded fertilizer levels *viz.,* 0, 25, 50 and 100 per cent recommended dose of fertilizer (RDF). The 12 treatment combinations were replicated thrice in a split plot design. The results revealed that irrigation with secondary treated waste water recorded significantly higher plant height (226.1 cm), leaf area index (11.93), NDVI (0.70), green fodder yield (488.4 q ha^{-1}), dry matter yield (103.7 q ha^{-1}), NPK uptake (147.2, 26.01 and 138.2 kg ha^{-1}, respectively) and higher B:C (2.25) and it was on par with tertiary treated waste water as compared to borewell water irrigation. Among fertilizer levels, application of 100 per cent RDF recorded significantly higher green fodder yield (589.1 q ha^{-1}), dry matter yield (127.5 q ha^{-1}), nitrogen uptake (193.8 kg ha^{-1}), phosphorus uptake (37.29 kg ha^{-1}) and potassium uptake (187.6 kg ha^{-1}) compared to lower fertilizer levels. Interaction of secondary treated waste water with 100 per cent RDF recorded higher green fodder yield (609.3 q ha^{-1}), dry matter yield (132.5 q ha^{-1}), crude protein content (9.66%), net returns (¹ 78,229 ha^{-1}) and benefit cost ratio (2.57). However, waste water irrigation exhibited a nutrient substitution effect, wherein 0 and 25 per cent RDF under secondary treated waste water produced yields comparable to 25 and 50 per cent RDF under borewell irrigation, respectively. Post-harvest soil physico-chemical properties and heavy metal concentrations remained within permissible limits and did not vary appreciably among the treatments.

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Natural Farming Studies In Redgram - Foxtail Millet Sequence

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A field experiment was conducted at RIOF, UAS, GKVK, Bengaluru during 2022-23 and 2023-24 to study the effect of natural farming systems on growth and yield of redgram-foxtail millet sequence. The experiment was laid out in RCBD design and replicated five times. The experiment consisted of 7 treatments (T_1: absolute control, T_2: organic production system, T_3: natural farming practice, T_4: natural farming+ FYM on N equivalent basis, T_5: combination of natural farming and organic farming practices, T_6: farmers practice and T_7: UASB POP. The pooled data clearly revealed that among the various farming practices significantly higher redgram seed yield (1265 kg ha^{-1}) and stalk yield (3343 kg ha^{-1}) was recorded in UASB POP compared to other treatments and was found on par with combination of natural farming and organic farming practices (1153 and 3261 kg ha^{-1}, respectively). Whereas, absolute control recorded significantly lowest yield (375 and 1685 kg ha^{-1}, respectively). Similar trend was noticed in foxtail millet also, where UASB POP recorded higher grain (1710 kg/ha) and straw yield (2831 kg/ha) and was found on par with combination of natural farming and organic farming practices (1537 and 2748 kg ha^{-1}, respectively). Higher net returns for redgram-foxtail millet sequence (Rs.48874 and Rs.27441 ha^{-1}, respectively) was recorded in the UASB POP. Thus, the UASB POP and combination of natural farming and organic farming practices were found productive and remunerative.

Influence of Organic Manure on Growth Yield and Quality of Browntop Millet (*Brachiaria ramosa* L.)

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A field experiment entitled 'Influence of organic manure on growth yield and quality of browntop millet (*Brachiaria ramosa* L.)' was conducted during *kharif* 2023 at College of Agriculture, V.C. Farm, Mandya. The experiment consisted of thirteen treatments involving 100%, 75%, 50% N equivalent application of farmyard manure, green leaf manure and bio digested liquid along with application of microbial consortia @ 5 kg ha^{-1}. The experiment was laid out in RCBD design along with three replications. The result revealed that application of 100% N equivalent bio digested liquid + microbial consortia @ 5 kg ha^{-1} recorded significantly higher plant height (80.97 cm), leaf area (557.41 cm^2 plant^{-1}), number of tillers plant^{-1} (5.98), root length (18.95 cm), root volume (9.54 cm^3), root density (0.96 g cm^3) and dry matter production and its distribution in leaf (5.05 g/plant), stem (5.80 g/plant), panicle (3.35 g/plant) and total dry matter (13.98 g/plant), number of panicles (41.29 m^{-2}), length of panicles (17.97 cm), number of seeds panicle^{-1} (115.61), grain weight panicle^{-1} (2.99 g), test weight (3.93 g), grain yield (1252 kg ha^{-1}), straw yield (1947 kg ha^{-1}) and harvest index (0.39). The quality parameters like crude protein content (11.55%), crude fiber content (12.38%), total CHO content (71.50%), ash content (5.55%), oil absorption capacity (8.75 ml g^{-1}), water absorption capacity (8.84 ml g^{-1}), cooking time (17.89 min) and solid loss (0.88%) was also significantly high in above treatment. The higher major nutrient uptake of N:P:K:S (57.64:15.27:35.32:9.23 kg ha^{-1}, respectively) and micro nutrient uptake of Zn:Mn:Fe:Cu (1343.42:103.6:2184.40:291.11 kg ha^{-1}, respectively), was recorded in the same treatment. The application of 100% N equivalent bio digested liquid + microbial consortia @ 5 kg ha^{-1} was found to enhance the yield with better net return (Rs.32046 ha^{-1}) and higher benefit cost ratio (2.16) in Browntop millet.

Studies on the Effect of Various Organic Nutrient Sources on Growth, Yield and Nutrient Dynamics in Maize

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A field experiment was conducted at the Research Institute on Organic Farming (RIOF) and Agronomy Field Unit, GKVK, UAS, Bangalore during *rabi* 2023 to study the effect of various organic nutrient sources on growth, yield and nutrient dynamics in maize using a randomized complete block design. The experiment included eleven treatments with three replications, applying various organic nutrient sources based on nitrogen equivalent, including farmyard manure, vermicompost, poultry manure, jeevamrutha, RDF and absolute control. Among various organic nutrient sources, poultry manure at 100% N equivalent recorded the higher plant height (215.4 cm), number of leaves plant^{-1} (12.86), leaf area (4093 cm^2 plant^{-1}), dry matter accumulation (110.5 g plant^{-1}), cob length (17.32 cm), kernel rows per cob (20.18), kernels per row (33.02), kernel weight per cob (156.16 g), test weight (25.23 g), kernel yield (86.76 q ha^{-1}) and stover yield (117.87 q ha^{-1}). It also resulted in the higher uptake of nitrogen (205.7 kg ha^{-1}), phosphorus (35.92 kg ha^{-1}) and potassium (168.4 kg ha^{-1}). The soil microbial activity *viz*., dehydrogenase, urease and phosphatase enzyme activities along with microbial biomass carbon and nitrogen were significantly enhanced with application of poultry manure at 100% N equivalent. Economic analysis showed that the higher net returns (Rs.1,39,108 ha^{-1}) and B:C ratio of 3.69, making poultry manure the most effective organic nutrient source. These results suggest that poultry manure is an excellent option for enhancing maize growth, yield, soil health and economic returns, promoting sustainable organic farming.

Studies on Green Manuring and Different Organic Sources of Nutrients on Soil Properties and Yield of Maize-Cowpea Cropping Sequence

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A field experiment was conducted at research and demonstration block of Research Institute on Organic Farming, UAS, GKVK, Bengaluru during 2022-23 and 2023-24 to study the effect of green manuring and different organic sources of nutrients on soil properties and yield of maize-cowpea cropping sequence. The experiment was laid out in a split plot design with three replications. The experiment included 18 treatment combinations which consisted of two main plots *i.e.,* with and without *in situ* incorporation of sunhemp as a green manure crop and nine subplots comprising three levels of N equivalent and four organic sources of nutrients. The soil of experimental site was red sandy loam having medium organic carbon (0.64 %), medium in available N (291.2 kg ha^{-1}), medium in available P_2O_5 (26.24 kg ha^{-1}) and medium in available K_2O (228.3 kg ha^{-1}) content. The experimental results indicated that *in situ* incorporation of green manure followed by the combined application of 25% N equivalent FYM + 25% N equivalent poultry manure + 25% N equivalent vermicompost + 25% N equivalent jeevamrutha for maize resulted in significantly higher plant height (204.1 cm), leaf area (7102 cm^2 plant^{-1}), total dry matter accumulation (163.4 g plant^{-1}), kernel yield (91.67 q ha^{-1}), stover yield (125.4 q ha^{-1}) and B:C ratio (3.10). In maize-cowpea cropping sequence, cowpea grown as a residual crop recorded significantly higher grain yield, haulm yield and B:C ratio (1386 kg ha^{-1}, 2903 kg ha^{-1} and 2.37) in *in situ* incorporation of green manure followed by the combined application of 25% N equivalent FYM + 25% N equivalent poultry manure + 25% N equivalent vermicompost + 25% N equivalent jeevamrutha. This combination was found to be significantly superior over other treatments in the studies.

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Effect of Nutrient Sources on Straw Yield and Quality of Finger Millet (*Eleusine coracana* L.) Varieties

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A field experiment entitled 'Effect of nutrient sources on straw yield and quality of finger millet (*Eleusine coracana* L.) varieties' was conducted at College of Sericulture, Chintamani during late *Kharif*, 2023. The experiment was laid out in Factorial Randomized Complete Block Design with two factors (varieties and nutrient sources) at three levels each resulting in 9 treatment combinations. Three varieties (ML 322, KMR 316 and KMR 630) were tested with nutrient sources (100 per cent RDF, 50 per cent RDN + 2 sprays of 4 per cent nano urea and 50 per cent RDN + 2 foliar sprays of 5 per cent jeevamrutha. The results revealed that variety ML 322 recorded significantly higher plant height (126.7 cm), leaf area (930.3 cm^2 $plant^{-1}$), total dry matter accumulation (41.8 g $plant^{-1}$), leaf to stem ratio (0.56), SPAD reading value (41.63), number of earheads per plant (3.02), grain yield (3600 kg ha^{-1}), straw yield (5438 kg ha^{-1}), crude protein content (2.97 %), crude fibre content (26.28 %), and total nitrogen uptake (70.70 kg ha^{-1}). Among the nutrient sources, application of 100 per cent RDF has recorded significantly higher plant height (125.6 cm), leaf to stem ratio (0.58), number of earheads per plant (3.04), grain yield (3557 kg ha^{-1}) and total nitrogen uptake (69.67 kg ha^{-1}). However, significantly higher leaf area (947.1 cm^2 $plant^{-1}$), total dry matter accumulation (41.9 g $plant^{1}$), SPAD reading value (36.55), straw yield (5367 kg ha^{-1}), crude protein content (2.97 %) and crude fibre content (26.30 %) was recorded in the treatment receiving 50 per cent RDN + 2 sprays of 4 per cent nano urea. Therefore, it is evident that basal application of 50 per cent RDN along with foliar application of nano urea twice results in higher straw yield and improved the straw quality.

Weed Management Practices for Enhancing Productivity of Direct Seeded Rice (DSR)

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A field experiment was conducted during *rabi* 2024 at Zonal Agricultural Research Station, University of Agricultural Sciences, Bangalore. Twelve treatments replicated thrice were laid out in a Randomized Complete Block Design (RCBD). Three pre-emergence herbicides (pendimethalin, pyrazosulfuron-ethyl and oxyfluorfen), three post-emergence herbicides (triafamone + ethoxysulfuron, bispyribac-sodium and penoxsulam + cyhalofop) and four intercropping treatments rice + French bean and rice +field bean in which two treatments received pendimethalin and the other two received oxyfluorfen were compared with passing cycle weeder and hand weeding at 15, 30 and 45 DAS and a weedy check. Passing cycle weeder and hand weeding at 15, 30 and 45 DAS has recorded significantly higher grain and straw yield (5477 and 7403 kg ha^{-1}) followed by triafamone + ethoxysulfuron 30% WG (RM) at 60 g a.i. ha^{-1} as EPoE fb CW & HW at 35 DAS (5317 and 7091 kg ha^{-1}). Higher net returns and B:C ratio was recorded in DSR + French bean intercropping (1:1) ratio with pendimethalin 38.7% CS at 677 g a.i. ha^{-1} as PE fb CW & HW at 45 DAS (Rs.177879 ha^{-1} and 4.43) followed by DSR + French bean intercropping (1:1) ratio with oxyflurofen 23.5 EC at 105.75 g a.i. ha^{-1} as PE fb CW & HW at 45 DAS (Rs.145239 ha^{-1} and 3.80). The unweeded control recorded significantly lower rice grain yield, net returns, and B:C ratio (1467 kg ha^{-1}, Rs.8761 ha^{-1} and 1.25, respectively) and exhibited a higher weed index (73.22%) compared to other treatments.

Response of Grain Amaranth (*Amaranthus hypochondriacus* L.) to Nano Urea and Nano Sulphur

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A field experiment entitled 'Response of grain amaranth (*Amaranthus hypochondriacus* L.) to nano urea and nano sulphur' was conducted at 'M' block, University of Agricultural Sciences, Bangalore during *kharif* 2024. The experiment consists of ten treatments which were replicated thrice in RCBD. Treatments comprised of varying levels of RDF (50, 75 and 100 %) and foliar application of nano urea and nano sulphur @ 4 ml L^{-1} at 30 and 45 DAS. Among the different treatments, application of 100 % RDF + 2 sprays of nano urea and nano sulphur recorded significantly higher growth, yield and yield parameters *viz*., plant height (169.1 cm), number of leaves $plant^{-1}$ (24.55), total dry matter production $plant^{-1}$ (104.3 g), panicle length (42.62 cm), number of fingers $panicle^{-1}$ (38.65), finger length (20.89 cm), grain yield (1815 kg ha^{-1}) and stover yield (6168 kg ha^{-1}) of grain amaranth over RDF alone. Notably, this particular treatment also proved the most financial rewarding by generating higher gross returns (Rs.145200 ha^{-1}) and net returns (Rs.109561 ha^{-1}). However, the higher B:C (4.15) was achieved in 75 % RDF + 2 sprays of nano urea and nano sulphur due to reduced input cost without a proportionate drop in yield contributed to better economic efficiency and outperforming all other treatment options. These findings emphasize the potential of nano urea and nano sulphur with conventional practices to optimize productivity and profitability of grain amaranth.

Agronomic Investigation on Fodder Quality and Productivity of Barnyard Millet (*Echinochloa frumentacea* L.)

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A field experiment was conducted at Zonal Agricultural Research Station, University of Agricultural Sciences, Bangalore during *kharif* 2024 to study the 'Agronomic investigation on fodder quality and productivity of barnyard millet (*Echinochloa frumentacea* L.)'. The experiment was laid out in RCBD with factorial concept, considered three factors: nitrogen levels (40, 60 and 80 kg N ha^{-1}), planting geometry (30 cm × 10 cm and 22.5 cm × 10 cm) and harvesting stages (earhead emergence and physiological maturity) with 12 treatment combinations replicated thrice. Among nitrogen levels, 80 kg N ha^{-1} recorded significantly higher green fodder yield (15,731 kg ha^{-1}), dry fodder yield (3,969 kg ha^{-1}), grain yield (2,376 kg ha^{-1}) and protein content (7.30%) with lower fibre constituents. Among planting geometries 22.5 cm × 10 cm produced higher green fodder yield (14,999 kg ha^{-1}) and dry fodder yield (3,601 kg ha^{-1}) while, 30 cm × 10 cm recorded higher grain yield (2,250 kg ha^{-1}). Planting geometry had no significant effect on fodder quality. Among harvesting stages, harvesting at earhead emergence recorded higher green fodder yield (16,263 kg ha^{-1}) and protein content (8.17%) with lower fibre constituents, whereas at physiological maturity recorded higher dry fodder yield (3,312 kg ha^{-1}). The crop harvested at physiological maturity as dual-purpose recorded higher gross returns (Rs.1,09,944 ha^{-1}) and net returns (Rs.71,809 ha^{-1}) under 80 kg N ha^{-1} with 30 cm × 10 cm spacing, while higher B:C (3.05) was obtained with 60 kg N ha^{-1} with 30 cm × 10 cm spacing.

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Calibration and Validation of Ceres-Maize Model for Hybrid Maize under Varied Irrigation and Nitrogen Levels

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A field experiment was conducted at ZARS, V.C. Farm, Mandya during *rabi* 2024 on 'Calibration and validation of CERES-maize model for hybrid maize under varied irrigation and nitrogen levels'. The experiment was laid out in split plot design with two irrigation levels as main plot and five nitrogen levels as sub plot, replicated thrice. Among irrigation levels, IW/CPE = 1.0 (I_2) recorded higher plant height (159.07 cm), Leaf area index (LAI) (2.41), Total dry matter production (TDM) (285.51 g plant^{-1}), kernel (5845 kg ha^{-1}) and stover yield (8984 kg ha^{-1}), but was at par with IW/CPE = 0.8 (I_1). Among nitrogen levels, plant height (200.41 cm), LAI (3.56), TDM (357.36 g plant^{-1}), kernel (8102 kg ha^{-1}) and stover yield (11,180 kg ha^{-1}) were significantly higher in 125% (N_5) and was at par with 100% RDN (N_4). CERES-Maize model was calibrated and validated by using data collected from the field experiments. The model satisfactorily predicted the TDM, LAI, number of kernels cob^{-1}, 100 seed weight, cob length, kernel and stover yield with RMSE of 40.52 g plant^{-1}, 0.65, 52.90, 2.03 g, 1.11 cm, 508.46 and 1150.80 kg ha^{-1}, respectively. The higher net returns (Rs.1,49,806 ha^{-1}) and B:C ratio (3.44) was recorded in I_1N_5 and I_1N_4, respectively. Hence, it is inferred that the CERES-Maize model was successfully validated for irrigation and nitrogen levels in Cauvery Command Area of Karnataka with model efficiency of 96 per cent and hence, model can be used as a tool for strategic decisions.

Morphometric and Molecular Characterization of *Apis cerana* F. Populations from Karnataka

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The present study titled 'Morphometric and molecular characterization of *Apis cerana* F. populations from Karnataka' was conducted during 2023–24 at Department of Apiculture, UAS, Bangalore. This study examines the morphometric and molecular diversity of *Apis cerana* worker populations (n=14) collected from 10 districts of 12 locations in Karnataka, focusing on two morphs *viz*., black and yellow. Morphometric analysis revealed significant morphological differences between morphs. Worker bees of black morph exhibited larger body lengths (11.30–14.85 mm, average 12.96 mm) compared to the bees of yellow morphs (9.91–13.66 mm, average 11.89 mm). Thorax length (3.92 mm), thorax width (3.46 mm), antenna length (3.86 mm), forewing length (8.43 mm) and hind leg length (9.46 mm), tibia length (2.71 mm), consistently showed higher values in black morphs. Molecular characterization using mitochondrial COI gene sequencing identified two phylogenetic clades. Clade I comprised black morph populations from Dharwad, Attigere (Chikmagalur) and Madenadu (Kodagu) clustering with reference sequences from Nagaland, Idukki and Wayanad. Clade II included yellow morph populations from Gokarna (Uttara Kannada), Pathaganahalli (Tumkur) and Kaggalipura (Bengaluru), clustering with sequences from Odisha. Genetic divergence ranged from 0.0000 to 0.0529, with the highest divergence observed between Pathaganahalli (Tumkur) and Madenadu (Kodagu). Yellow morph populations exhibited a higher A+T nucleotide composition (75.59%) than black morph populations (74.21%). The study highlights the effectiveness of integrating morphometric and molecular approaches to understand phenotypic and genetic variability in *A. cerana*. These findings offer insights into the diversity of this species, aiding in the development of selective breeding programs and supporting sustainable apiculture in Karnataka.

Nutritional Evaluation of Microgreens and its Exploration into Value Added Products

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MICROGREENS, the nutrient-dense shoots of vegetables and herbs are gaining popularity for their superior nutritional and bioactive potential. This study, 'Nutritional evaluation of microgreens and their exploration into value-added products', assessed the growth, composition, bioactive properties and product development potential of Red Amaranthus, Fenugreek, and Spinach microgreens through a standard protocol. Microgreens grown in a cocopeat + vermicompost medium, achieved optimal growth with fenugreek reaching the highest yield (203.66 g fresh weight, 11.44 g dried weight). Drying the microgreens at 40°C retained the highest Total Polyphenols Content (TPC), with Red Amaranthus, Fenugreek, and Spinach recording 186.91 mg GAE/g, 61.49 mg RE/g and 173.85 mg GAE/g, respectively. Vitamin C retention was highest in fenugreek (46.55 mg/100 g), followed by red amaranthus (45.44 mg/100 g) and Spinach (32.32 mg/100 g). Microgreens exhibited higher mineral content than mature greens, with Red Amaranthus containing 733.78 mg magnesium, 365.59 mg calcium, and 253.84 mg potassium. Bioaccessibility was significant, with calcium in Spinach (38.08%) and magnesium in Red Amaranthus (38.25%). Anti-nutritional factors like oxalic acid were lower than in mature greens. Value-added products such as sambar powder, fryums, and *tokku* were developed and organoleptic qualities were assessed by 9 point hedonic scale. Red Amaranthus sambar powder (80%) scored 8.50 in acceptability, while Fenugreek and Spinach fryums (30% and 20%) scored 8.70 and 8.59, respectively suggesting highly acceptability. *Tokku* with 75% Red Amaranthus and 100% Fenugreek achieved the highest scores (8.70 and 8.80). This study underscores the immense potential of microgreens in enhancing nutritional intake and creating innovative, health-focused food products.

Processing and Development of Value Added Food Products from Air Potato (*Dioscorea bulbifera*)

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AIR POTATO (*Dioscorea bulbifera*) is a twining herbaceous plant that attain heights of 25-30 meters. It's a nutrient-rich plant with higher carbohydrate and mineral content. Air potatoes are being underutilized, hence the current study 'Processing and development of value added food products from air potato (*Dioscorea bulbifera*)', was conducted to explore it's potential. Fresh air potato bulbils displayed significant physical characteristics. Various pretreatments were carried out to reduce browning reaction and analyzed for their effects on dehydration and rehydration ratios. Potassium metabisulfite (KMS) pretreatment significantly reduced browning reaction, yielding the highest rehydration ratios. The air potato flour demonstrated favorable physico-chemical, functional properties with notable antioxidant activity. Air Potato Flour (APF) was primarily composed of carbohydrates (84 gm), total ash (3.72 gm) with 355 kcal of energy per 100 gm. APF had minerals such as potassium (338.48 mg), calcium (315. 80 mg), magnesium (119.12 mg), phosphorus (138.06 mg) and iron (12.36 mg) per 100 gm. Innovative food products such as crackers, soup mix and gravy premix incorporated with air potato flour were developed. Sensory evaluations indicated that crackers containing 30 per cent, soup mix containing 80 per cent and gravy premix with 10 per cent of air potato flour achieved the highest overall acceptability.The storage period affected moisture, free fatty acids, peroxide value and microbial load but all levels were within permissible limit. The study also evaluated that packaging materials found that metallized polypropylene (MPP) and aluminum (ALM) foil provided superior barrier properties up to 45 days of storage. Overall, the findings revealed the versatility of air potato as a valuable ingredient for enhancing nutritional quality in food products.

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Technological Optimization of Fruit-Based Cake Premixes

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CAKE premix is a mixture containing all the dry ingredients used for preparation of cakes. Cake premixes are available in different flavours such as vanilla, chocolate, plum, pineapple, strawberry, etc. These cake premixes can be enriched with dehydrated fruit flours to improve the nutritional profile of the cakes. Hence, the present study was undertaken to formulate the fruit-based cake premixes with incorporation of the dehydrated fruit flours. Ripe banana fruit flour and ripe jackfruit flour were incorporated to prepare banana flour-based cake premix (BFCP) and jackfruit flour-based cake premix (JFCP), respectively. The study revealed that cakes prepared using BFCP with 15 per cent banana flour and JFCP with 20 per cent jackfruit flour had highest overall acceptability scores. The vitamin analysis of cakes revealed significant differences among the control, banana flour-based cake (BFC), and jackfruit flour-based cake (JFC). BFC and JFC exhibited higher levels of vitamin C (1.01 mg and 2.02 mg, respectively) and β-carotene (8.72 µg and 4.95 µg, respectively) compared to the control (0.34 mg and 1.01 µg, respectively). The total phenolic content of BFC and JFC was 73.68 mg GAE and 113.6 mg GAE, respectively as compared to 2.69 mg GAE for control cake. Total flavonoid content was also significantly higher in BFC (23.04 mg CE) and JFC (32.95 mg CE) as compared to the control (0.94 mg CE). Consumer acceptability survey showed, the highest acceptability of 94 per cent for jackfruit flour-based cake, 82 per cent for banana flour-based cake and 85 per cent for control. Thus, the study demonstrated that dehydrated fruit flours can be efficiently used in cake formulations to improve the nutrient profile of the cakes using cake premixes.

Processing and Development of Value Added Products from Hog Plum (*Spondias mombin*)

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HOG PLUM (*Spondias mombin*) is a underutilized nutrient-dense fruit with high antioxidant content and therapeutic properties. The present study was undertaken to study the processing and development of value-added products from Hog Plum. Hog Plum fruit had an average weight, length, fruit-to-seed ratio, peel-to-pulp ratio and specific gravity of 37.07± 2.54g 44.15± 5.11mm, 6.54 ± 0.45, 60.57 ± 0.08 and 1.35, respectively. Pre-treatment with 1% KMS followed by drying at 60°C produced Hog Plum powder with superior quality, yielding 11.8 g of flour per 100 g of fresh fruit. Hog Plum powder exhibited high solubility (97.87%) and dispersibility (87.04 s). Hog Plum powder per 100g contained 8.37 ± 0.36 per cent moisture, 1.47 ± 0.12g protein, 0.27 ± 0.05g fat, 0.61 ± 0.05g ash, 13.80 ± 0.03g carbohydrate and 63.51 ± 0.03Kcal. Micronutrient composition of it includes calcium, potassium, phosphorus, magnesium, sodium, manganese manganese, copper, iron and zinc 302 mg/100g, 248 mg/100g, 29.6 mg/100g, 10 mg/100g, and 3.6 mg/100g, at 0.29 mg/100g, 0.12 mg/100g, 0.94 mg/100g and 0.23 mg/100g, respectively and exhibited good antioxidant level *viz.,* 47.33mg/100g vitamin C and 26.19mg/100g polyphenol. Among the developed products 100 per cent incorporated Hog Plum *chutney* powder, pickle and juice scored best (8.7, 8.3 and 8.3), whereas *puliyogare* mix and marmalade were best accepted at 50 and 75 per cent, respectively. The developed products were safe for 90 days when packed in suitable packaging material at ambient temperature. Thus, one can exploit the health benefits of Hog plum by processing and value addition.

A Gender-Based Study on Body Composition and Lifestyle Patterns among Bus Conductors in Bengaluru

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Bus conductors form an essential component of Bengaluru public transportation system however, demanding work schedules, irregular meal timings, prolonged standing hours and continuous exposure to occupational hazards place them at increased health risk. This study assessed socio-demographic profile, dietary pattern, nutrient adequacy, lifestyle practices, body composition and occupational health hazards among 100 BMTC bus conductors (50 males and 50 females) selected through simple random sampling. Data were collected using semi-structured questionnaire. Anthropometric and body composition parameters were assessed using standard tools such as measuring tape, skinfold calipers and Tanita Body Composition Analyzer (RD-953). Socio-demographic findings showed that females predominantly belonged to younger age group (58%), whereas males were mainly older age groups (42%). Joint families were more common among females (76%). Males had longer occupational experience. Significant gender differences were observed in nutritional and body composition indicators. Female conductors showed higher prevalence of pre-obesity (38%) and obesity (32%), whereas 40 per cent of males had normal BMI. WHR classification indicated higher risk of central obesity among males (84%) compared to females (42%). Macronutrient adequacy was higher among males (protein 116%, fat 140%). Females exhibited greater micronutrient inadequacies, particularly iron (44.4%) and calcium (61.5%). Majority of respondents had low total body water levels, indicating chronic dehydration and restricted breaks during duty hours. Lifestyle assessment revealed inadequate sleep (66-72%), universal rotating shifts and limited leisure time physical activity. Non-food habits were prevalent among males, *viz.,* tobacco chewing (74%), smoking (62%) and alcohol consumption (74%). Occupational hazards were widespread with all respondents reporting stress and 86-100 per cent experiencing environmental exposures. The findings highlights the need for targeted gender sensitive workplace interventions focusing on nutrition, hydration and stress management to enhance the overall health and occupational well-being.

Development of Seed Production Technology to Enhance Seed Yield and Quality in Maize (*Zea mays* L.) Hybrid MAH 15-84

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This study aimed to standardize seed priming techniques and evaluate their impact on seed quality, flowering synchronization, growth and seed yield characteristics of the parental lines of maize hybrid MAH 15-84 (*Zea mays* L.). Laboratory experiments were conducted at the Department of Seed Science and Technology, GKVK, Bengaluru during 2022 involved seed priming of male (MAI-19-20) and female (MAI-19-117) parents with KNO_3, $ZnSO_4$, GA_3, salicylic acid and hydropriming for 12 and 24 h with unprimed seeds as control. Results revealed that, priming with 50 ppm GA_3 for 12 h (T_9) significantly enhanced the seed germination (99.33 and 99.00 %), root length (29.70 and 27.33 cm), seedling dry weight (93.31 and 92.33 mg), seedling vigour index-I (5459 and 5282), seedling vigour index-II (9268 and 9141) while reducing electrical conductivity (0.262 and 0.228 dSm^{-1}) and radicle emergence time (70.93 and 69.07 h). Field experiments during *rabi* 2022 and *kharif* 2023 evaluated the effects of seed priming (S_6-50 ppm GA_3 for 12 h) and planting ratios (P_1-3:1 and P_2-4:1) on growth and seed yield. The P_1S_6 treatment (3:1 + priming to both parents) recorded the highest plant height (45.70, 179.00 and 190.90 cm at 30, 60 DAS and harvest, respectively), early tassel (53.16 days) and silk initiation (55.58 days), reduced anthesis-silking interval (2.41 days). It also improved cob length (15.24 and 16.52 cm), seed yield per cob (92.69 and 107.9 g) and seed yield per hectare (22.11 and 50.22 q/ha) in male and female, respectively. Genetic purity testing using SSR markers identified umc1152, bnlg1371, bnlg1043, umc2071 and bnlg107 as polymorphic markers for hybridity confirmation. The study concluded that seed priming with 50 ppm GA_3 for 12 h combined with 3:1 planting ratio optimizes seed quality, flowering synchronization and seed yield in maize hybrid MAH 15-84, particularly during the *rabi* season.

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Studies on Detection of Seed Borne Diseases and their Management in Chilli (*Capsicum annuum* L.)

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CHILLI fruit rot is a complex disease in chilli caused by *Colletotrichum truncatum, Colletotrichum gleosporioides, Colletotrichum acutatum, Alternaria alternata, Fusarium oxysporum* and *Fusarium sporotrichoides* which are seed borne and leads to seed deterioration, damping off and dieback causing heavy losses. Hence, identifying effective detection methods for seed-borne pathogens is crucial. This study experimented various methods to detect *Colletotrichum truncatum*. Among ten different seed health testing methods in detecting the seed borne infection of *Colletotrichum truncatum* in chilli, 19.65 per cent infection was observed with visual examination, while it was 31.48 per cent with microscopic examination and agar plate method recorded 37.75 per cent infection. PCR method showed 100 per cent seed infection with ITS primer amplifying a 560 bp fragment, confirming *Colletotrichum truncatum* presence in seeds. While evaluating the pathogenicity of *Colletotrichum truncatum* on seed quality parameters, least per cent seed infection of *Colletotrichum truncatum* was recorded in surface sterilized apparently healthy seed sample (0.00%), with highest germination (81.25 %), mean seedling length (8.43 cm), mean seedling dry weight (19.54 mg), seedling vigour index-I of 685 and seedling vigour index-II of 1588. Whereas, for managing chilli anthracnose, seed treatment with *Pseudomonas fluorescens* @ 6g/kg of seeds resulted in maximum plant height at harvest (51.00 cm) and was on par with Carbendazim 12% + Mancozeb 63% WP at 0.2 per cent (50.00 cm) and garlic blubs extract at 10 per cent (48.33 cm). While, maximum fruit yield (g/ plant) (149.60 g) and seed yield per plant (1.81 g) was obtained with resultant crop from seeds treated with Carbendazim12% + Mancozeb 63% WP at 0.2 per cent.

Standardization of Heat Stress Mitigation on Seed Yield and Quality in Soybean [*Glycine max* (L.) Merr.]

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SOYBEAN [*Glycine max* (L.) Merr.] a key oilseed and protein-rich crop holds immense importance in India's agricultural economy, serving as a vital source for edible oil. High-temperature stress adversely affects soybean growth, seed yield and quality by disrupting physiological and biochemical processes. Phytohormones and antioxidants mitigate heat stress by regulating growth, enhancing tolerance and minimizing oxidative damage. This study evaluated the effect of heat stress mitigation on the seed yield and quality of soybean, using eight different treatments applied at four different growth stages during 1st week of January and February, 2024 sowings in a strip plot design. The results showed that, salicylic acid @ 50 ppm sprayed at vegetative and flowering stages was recorded the highest plant height (62.10 cm and 61.23 cm), number of pods (75.51 and 71.55), seed yield per plant (26.28 g and 24.82 g), seed yield per hectare (38.94 q and 36.77 q), oil content (19.63 and 19.06 %), protein content (43.31 and 41.98 %), peroxidase activity (0.88 and 0.83 A_{436}) with highest B:C ratio (3.94 and 3.72), respectively, whereas, gibberellic acid at 100 ppm sprayed during vegetative and flowering stages showed the lowest seed moisture content (9.03 and 9.32 %) and highest seed germination (97.00 and 93.50 %) for 1st week of January and February, 2024 sowings, respectively. 1st week of January sowing performed better than 1st week of February sowing in all parameters, demonstrating the combined impact of treatment, growth stage and sowing time on mitigating heat stress and enhancing soybean productivity. In conclusion, salicylic acid @ 50 ppm, especially during the vegetative and flowering stages, improves growth, yield, seed quality and benefit-cost ratio under heat stress, making it an effective strategy for mitigating high-temperature stress in soybean.

Evaluation of Seed Borne Fungal Endophytes for Seed Quality Enhancement in Maize (*Zea mays* L.) and Chickpea (*Cicer Arietinum* L.)

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SEED bio-priming is an environmentally friendly technique that enhances seed germination, seedling vigor and plant resilience. This study investigated the effects of fungal endophyte biopriming on chickpea (JG-11) and maize (MAH 14-5) using five endophytes (L-20-1, L312, L35, L38-1 and L38-2) at three inoculum loads (2×10^5, 2×10^7, and 2×10^9 spores/mL) and four treatment durations (1, 2, 3 and 4 hour). Germination percentage, mean germination time, shoot and root length, seedling dry weight and vigor indices (SVI I and SVI II) were assessed. Results showed that *Nigrospora sphaerica* (L35) and *Curvularia hawaiiensis* (L38-1) at 2×10^7 spores/mL significantly improved germination and seedling growth, with optimal results at 3-hour and 4-hour treatment durations. Chickpea exhibited the highest vigor indices (SVI I: 5606, SVI II: 26704), while maize showed improved shoot length (21.4cm) and root length (30.5cm). Biochemical analyses revealed enhanced chlorophyll content, antioxidative enzyme activity and phytohormone production, leading to better seedling establishment. Endophyte biopriming demonstrated its potential as a sustainable alternative to chemical treatments by improving early seedling growth and plant resilience to environmental stress. These findings highlight the effectiveness of fungal endophytes in promoting plant development, offering an eco-friendly approach to enhance crop productivity while reducing chemical dependency in modern agriculture.

Evaluation of Seed Quality Attributes and Storage Potential of Bio-Fortified Hybrid in Maize (*Zea mays* L.)

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THE laboratory experiment was conducted at STR, AICRP on Seed, NSP, University of Agricultural Science, GKVK, Bengaluru during *kharif* 2023-24. The experiment consists of five QPM hybrids. The hybrids included IQPM-H-2001, HQPM-1 (Lot-1), HQPM-1 (Lot-2), HQPM-5 and MAH 14-5. Seeds were subjected to four treatments: Spinosad at 0.04 ml/kg, Neem seed kernel powder at 10g/kg, Pongamia leaf powder at 50g/kg and an untreated control. For storage, two packing materials were used: cloth bags and HDPE (High-Density Polyethylene) bags**.** Seed quality parameters were assessed using factorial completely randomized design and recorded seed quality parameters bimonthly. Among the hybrids, MAH 14-5 consistently displayed superior average seed quality. It exhibited the highest seed moisture content (9.66%), seed germination (87.83%), mean seedling length (31.77 cm), mean seedling dry weight (72.81 mg) and seedling vigour Index-I (2870), alongside the lowest seed infestation (2.20%). While HQPM-1 (Lot-1) had a higher protein content (10.43%), MAH 14-5 still outperformed it in seedling vigour Index-II, total dehydrogenase activity, and electrical conductivity. Regarding treatments, Spinosad at 0.04 ml/kg yielded the best results across most parameters, including seed moisture (8.42%), seed germination (83.29%) and protein content (10.70%), while significantly reducing seed infestation (2.04%). This was closely followed by neem seed kernel powder. Crucially, HDPE bags proved to be the more effective storage material, maintaining better seed moisture (8.84%), seed germination (80.83%) and overall seedling vigour compared to cloth bags. The study concluded that treating QPM hybrid seeds with Spinosad (0.04 ml/kg) and storing them in HDPE bags is the preferred method for long-term preservation of seed quality.

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Influence of Microbial Consortium and Nutrient Management on Seed Yield and Quality in Cowpea (*Vigna unguiculata* L.)

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IMPROVING seed yield and quality in leguminous crops like cowpea (*Vigna unguiculata* L.) is essential for achieving sustainable agricultural productivity. Integrated nutrient management, involving the combined use of chemical fertilizers and beneficial microorganisms, has emerged as a promising strategy to enhance both yield and seed quality. In this context, a field experiment was conducted to study the influence of microbial consortia and nutrient management on seed yield and quality in cowpea during *kharif* 2024 at AICRP on Arid Legumes, GKVK, UAS, Bangalore. To experiment consisted of ten treatments which were replicated thrice in Randomized Complete Block Design (RCBD). Among all treatments, the application of 100% RDF+ seed treatment with microbial consortia and soil application of microbial consortia significantly higher plant growth, seed yield and seed quality attributes. This treatment recorded the highest plant height (38.16 cm), number of root nodules (37.90), dry weight of root nodules (0.73 g plant^{-1}), number of pods plant^{-1} (13.83), pod length (18.16 cm), number of seeds pod^{-1} (15.96), hundred seed weight (12.33 g) and seed yield (18.14 q ha^{-1}). Further soil microbial count (42.77×10^{-}v , 18.37×10^{-4}, 19.52×10^{-3}, 26.17×10^{-5}, 22.97×10^{-5} CFU g^{-1} soil of bacteria , fungi, actinomycetes, free living N_2-fixer and PSB, respectively), seed germination (93.00%), seed vigour index-I (3815), protein content (25.93%), peroxidase activity (0.34 A_{436}), total dehydrogenase activity (2.93 A_{480}) and higher net return (67,808 Rs.ha^{-1}) and benefit cost ratio (2.5). Hence, it can be concluded that integration of microbial consortia with 100% RDF not only enhanced seed yield and quality in cowpea but also contributed to improved soil health and economic returns.

Enhancement of Mulberry Leaf and Silk Cocoon Productivity through Sensor-Based Precision Farming

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THE research entitled 'Enhancement of mulberry leaf and silk cocoon productivity through sensor-based precision farming' was conducted at the Department of Sericulture, UAS, GKVK, Bengaluru during 2022-23. There were nine treatment combinations comprising of different irrigation and nitrogen management methods which were replicated three times and laid out in strip plot design. The results revealed that sensor-based drip irrigation with normalised difference vegetation index (NDVI) based nano urea application recorded higher shoot length (54.61, 105.37 and 119.06 cm at 30, 45 and 60 DAP, respectively), number of leaves plant^{-1} (144.92, 305.93 and 334.76 at 30, 45 and 60 DAP, respectively), leaf area plant^{-1} (74.18, 131.72 and 166.07 cm^2 at 30, 45 and 60 DAP, respectively) and highest leaf yield (718.20 g plant^{-1} at 60 DAP). Similarly, significantly higher total leaf chlorophyll content (2.60 mg g^{-1}), moisture retention capacity after 6 hrs (82.40%) and nitrogen content (3.30%) were recorded compared to other treatment combinations at 45 DAP, whereas lower leaf yield and quality parameters were observed under yellow soil moisture indicator-based irrigation with no nitrogen application. Subsequently, there was positive response of double hybrid silkworm FC1 x FC2, when fed with mulberry leaves raised with different irrigation, nitrogen management and rearing methods. Significantly, higher V instar larval weight (42.53 g/10 larvae and 47.51 g/10 larvae), cocoon yield (21.90 kg/10,000 larvae and 24.76 kg/10,000 larvae), shell ratio (21.99 and 22.96%) and filament length (1273.88 m and 1317.38 m) were observed from the silkworm batches reared under sensor based controlled rearing environment and batches fed with mulberry leaves raised under sensor-based drip irrigation with NDVI based nano urea application, respectively. Similarly, maximum net returns (Rs.3,37,617 ha^{-1} yr^{-1}) with higher B:C ratio (2.83) was recorded in sensor-based drip irrigation with NDVI based nano urea application in mulberry and sensor-based silkworm rearing.

Efficacy of Silkworm Pupal Chitosan against Bacterial Diseases of Silkworm, *Bombyx mori* L.

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THE research was conducted to evaluate the efficacy of silkworm pupal chitosan against bacterial diseases of silkworm, *Bombyx mori* L. during 2024-2025 at Department of Sericulture, College of Sericulture, Chintamani. The results of the study revealed that, the chitosan exhibited significant antibacterial activity against *Staphylococcus aureus* (1.19 cm) and *Bacillus thuringiensis* (1.10 cm) with the higher inhibition zones observed at 5000 ppm compared to the standard ampicillin and commercial chitosan @ 1000 ppm in the *in-vitro* conditions. In the silkworm rearing studies, better larval weight (33.35 g/10 Nos.), cocoon weight (1.64 g), shell weight (0.32 g), shell ratio (19.57%), effective rate of rearing (73.56%), disease incidence (24.51%), pupation (76.30%) and moth emergence (81.98%) were observed in the silkworms treated with chitosan @ 5000 ppm by supressing *S. aureus* and *B. thuringiensis* action. The improvement in reeling parameters such as, higher filament length (936.41 m), filament weight (0.27 g) and filament denier (2.60) were also noticed in chitosan @ 5000 ppm among the silkworms infected with bacterial pathogens. Predominantly, standard treatments were recorded better over silkworm pupal chitosan treatments against *S. aureus* than *B. thuringiensis* in almost all the parameters. Early application of chitosan (6 hpi) resulted in better outcomes than delayed application (30 hpi). The study also indicated that, biochemical parameters such as protein, carbohydrate, protease and amylase levels in chitosan-treated silkworms increased daily, compared to those in bacteria-uninoculated controls, leading to reduced disease severity, while in silkworm batches inoculated only with bacteria, these contents decreased over time. Lipid content significantly declined in bacterial-infected silkworms as the disease progressed, while lipase activity fluctuated throughout the instar. The research suggests that, application of silkworm pupal chitosan at 5000/ ppm within 6/ hpi of bacterial inoculation is an effective measure for mitigating bacterial infections in silkworms, especially against *S. aureus* and *B. thuringiensis*.

Effect of Mulberry Biochar Application at Different Soil Depths on Mulberry Leaf and Cocoon Production

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A study on 'Effect of mulberry biochar application at different soil depths on mulberry leaf and cocoon production' was conducted in the established V-1 tree mulberry garden at KVK, Hassan during 2023. Experiment comprised of 10 treatments replicated thrice in RCBD. The experimental results revealed that combined application of 100 per cent RDF+FYM @ 10 t ha^{-1} + Biochar @ 15 t ha^{-1}/yr^{-1} at 30 cm soil depth significantly enhanced the growth, yield, biochemical parameters and elemental compositions of mulberry leaves. The same treatment significantly enhanced the soil water holding capacity (48.34%), pH (6.85), electrical conductivity (0.23 dS m^{-1}), primary, secondary and micronutrients content of the soil compared to 100 per cent RDF+FYM @ 10 t ha^{-1} + Biochar @ 15 t ha^{-1}/yr^{-1} at 15 cm soil depth T_4. Further, feeding mulberry leaves obtained by application of 100 per cent RDF+FYM @ 10 t ha^{-1} + Biochar @ 15 t ha^{-1}/yr^{-1} at 30 cm soil depth recorded significantly shorter fifth instar larval duration (7.30 days), higher fifth instar larval weight (54.20 g/10 larvae), effective rate of rearing (94.16%), cocoon weight (2.62 g), cocoon shell weight (0.58 g), pupal weight (2.03 g), filament length (1499.06 m) and filament weight (0.48 g) has compared to 100 per cent RDF+FYM @ 10 t ha^{-1} + Biochar @ 15 t ha^{-1}/yr^{-1} at 15 cm soil depth T_4.

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Integrated Management of Root Rot Disease in Mulberry under Field Conditions

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ROOT ROT disease poses a serious threat to mulberry (*Morus alba* L.) cultivation, leading to progressive wilting, poor leaf quality and reduced yield, thereby affecting the sericulture industry. The present study was undertaken to identify the causal organisms of root rot and to formulate an effective integrated disease management (IDM) strategy under field conditions. The root samples were collected from infected mulberry gardens in major mulberry-growing areas of Kolar and Chikkaballapur districts. The pathogens *Fusarium solani* and *Lasiodiplodia theobromae* were consistently isolated and confirmed through morphological characteristics and pathogenicity tests. A series of *in vitro* evaluations were conducted to assess the efficacy of various fungicides, bioagents and botanical extracts. Among systemic fungicides, Tebuconazole 25% WP and Propiconazole 25% EC achieved complete inhibition (100%) of mycelial growth, followed by the combi fungicide Carbendazim 12% + Mancozeb 63%WP. Garlic extract at 15 and 20 per cent showed strong antifungal activity among botanicals, while *Trichoderma harzianum* (Th-3 and Th-2) displayed superior antagonistic potential among the bioagents tested. Field experiments laid out in a randomized block design with eleven treatments, confirmed that Tebuconazole 25% WP recorded the lowest disease incidence (4.63%) and the highest leaf yield (1,934.92 g plant^{-1}), resulting in 81.48 per cent disease reduction over the control. Propiconazole 25% EC, Carbendazim 12% + Mancozeb 63%WP, garlic extract and *T. harzianum* were also significantly reduced the disease incidence and improved plant growth and yield. The study concluded that integrating systemic fungicides such as Tebuconazole 25% WP (1.00 ml/l) or Propiconazole 25% EC (1.00 ml/l) with *T. harzianum* (Th-3 or Th-2), garlic extract (15 or 20%) along with good field sanitation practices provides an effective, sustainable and eco-friendly approach for managing mulberry root rot under field conditions.

Impact of P Enriched Compost on Productivity of Finger Millet (*Eleusine coracana* L. Gaertn.) - French Bean (*Phaseolus vulgaris* L.) Cropping System in P Rich Soils

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FIELD experiment entitled as phosphorus enriched compost on the soil properties, growth and yield of the finger millet (*Eleusine coracana*) and French bean (*Phaseolus vulgaris*) cropping system in phosphorus rich soils was conducted at two locations in Karnataka, Arasana Halli, Chikkaballapur (L_1) and integrated farming system, GKVK, Bengaluru (L_2), during the *kharif* and summer of 2018-19 and 2023-24, respectively. Prior to initiation of the field experiment, four different composts *viz*., urban solid waste compost (USWC), poultry manure, FYM and vermicompost were enriched with 5 kg rock phosphate and 1 kg PSB were added to each 100 kg of compost. The experimental design adopted was RCBD with 14 treatments and replicated thrice. After enrichment of compost, bulk density (BD) and organic carbon (OC) was decreased, while all nutrients increased particularly phosphorus, which increased from 0.92 to 3.04 per cent in USWC and from 1.66 to 3.81 per cent in poultry manure. The results revealed that application of recommended N and K + 75 per cent P through enriched poultry manure (T_{11}) recorded higher number of fingers per head, test weight, straw yield (60.62 and 61.83 q ha^{-1}) and grain yield (43.57 and 45.83 q ha^{-1}), available N (153.87 and 281.01), P_2O_5 (84.46 and 76.04) and K_2O (132.34 and 252.43 kg ha^{-1}) of finger millet at L_1 and L_2, respectively. In French bean cropping system, T_{11} recorded higher fresh pod yield (28.25 and 27.10 t ha^{-1}) and haulm yield (2867 and 2868 kg ha^{-1}) and soil available N (111.30 and 236.47 kg ha^{-1}), P_2O_5 (69.37 and 57.77 kg ha^{-1}) and K_2O (133.21 and 184.65 kg ha^{-1}) at L_1 and L_2, respectively. After the harvest of both crops, T_{11} recorded higher OC, pH, BD, MWHC, dehydrogenase activity and P fractions in soil were higher in Finger millet and French bean cropping system at both the locations.

Studies on Silkworm Pupa Composting and its Influence on Soil Properties, Growth and Yield of Maize-Cowpea and Finger Millet-Cowpea Cropping System

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THE preparation of silkworm pupa compost (SPC) was standardized using different combinations of FYM and silkworm pupa (2:1 and 4:1 ratios) with and without the addition of waste decomposer (@ 5 kg and 10 kg/ton). Total N (1.90 %), P (0.96 %), K (1.51 %), Ca (2.32 %), Mg (1.29 %) and micronutrients increased over the period of decomposition in C5 treatment which received partially decomposed farm waste + silkworm pupae (2:1) and waste decomposer (10 kg/t) besides resulting in higher enzymatic activities and microbial population. Further, Studies on nutrient release pattern of SPC were conducted through a 90-day laboratory incubation in red sandy loam soil with neutral pH. The treatments included control (only soil) and soil amended with different compost formulations (C1 to C5) each applied at 10 t/ha. Nitrogen levels increased steadily from 133.46 to 175.93 mg/kg, phosphorus availability rose from 8.05 to 14.72 mg/kg, and potassium content improved from 80.10 to 111.46 mg/kg in the treatment amended with C5 compost (T_7). A field study was laid out in a randomized complete block design with three replications to assess the impact of SPC and FYM (10 t/ha) combined with 100% and 75% RDF on maize, finger millet and residual cowpea. The highest growth and yield were observed in T_{11} (100% RDF + C5 @ 10 t/ha) followed by T_7 (100% RDF + C3 @ 10 t/ha) in both maize and finger millet crops. Treatment T_{11} exhibited higher nutrient content, greater nutrient uptake, enhanced enzymatic activity and increased microbial population resulting in higher maize kernel (84.15 q/ha) and stover (92.81 q/ha) yield, while finger millet yielded 33.73 q/ha of grain and 46.09 q/ha of straw. The higher performance of cowpea was observed in the same treatment indicating the best possible use of silkworm pupa waste and proper composting.

Effect of PSB-Zn Enriched Coconut Shell Biochar on Phosphorus and Zinc Nutrient Dynamics, Soil Properties and Productivity of Soybean (*Glycine max* L.) in Acidic Soil

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BIOCHAR, a stable carbon-rich material produced via pyrolysis of organic waste, is highly beneficial for ameliorating acidic soils by raising soil pH and reducing exchangeable aluminium. In this context, field experiments were conducted at ICAR-KVK, Hadonahalli, Doddaballapura during the *kharif* seasons of 2022 and 2023 to study the effect of enriched biochar on phosphorus and zinc nutrient dynamics, soil properties and productivity of soybean under acidic soil conditions. The experiment comprised of thirteen treatments laid out in a randomized complete block design with three replications. The pooled data over two years revealed significant improvements in soybean growth and yield parameters. Enhanced plant height, number of pods and seeds per plant were observed. Notably, the treatment (T_6) receiving 100% NPK along with PSB enriched FYM at 3.125 t ha^{-1} and 100% Zn enriched biochar at 5 t ha^{-1} recorded the highest seed yield (23.61 q ha^{-1}) and biomass yield (2.83 t ha^{-1}), significantly outperforming other treatments. Among the treatments, T_5 (RDF + PSB enriched biochar at 10 t ha^{-1}) significantly improved soil physical properties, including maximum water holding capacity (44.68%) and bulk density (1.33 Mg m^{-3}), compared to T_2 (POP) and control (T_1). Improved soil chemical properties including increased organic carbon (0.61%), pH (6.26) and reduced acidity in enriched biochar treated plots were also recorded. Pooled data indicated significant increases in available phosphorus and zinc with PSB and Zn enriched biochar with T_6 consistently recording higher nutrient availability across all growth stages. Nutrient use efficiency also varied with phosphorus and zinc efficiency at 18.59 and 1.51 per cent, respectively, under T_6. The combined application of PSB enriched FYM and Zn enriched biochar effectively reduced soil acidity, minimized phosphorus fixation by aluminium and iron and demonstrated strong potential to enhance soil health, nutrient availability and soybean productivity in acidic soils.

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Studies on Nutrient Management Practices on Growth and Yiled of Small Onion (*Allium cepa* L. Var. *aggregatum*)

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A field experiment was conducted during *rabi* 2023 at Krishi Vigyan Kendra, Haradanahally Farm, Chamarajanagara to evaluate the nutrient management for Small onion. The experiment comprised of nine treatments replicated three times using RCBD. The treatments included application of 100, 75 and 50% RDF, in comparison to 100, 75 and 50%. RDN:K:S by varying RDP at 125 and 150%. Application of 100% RDN:K:S with 150% RDP (T_7) were recorded higher yield (21.64 ton ha^{-1}) and B:C ratio (4.10). Which was on par with application of 100% RDF with plant height (48.74), polar diameter (3.53cm), number of bulbs per clump (8.92), yield (20.76 ton ha^{-1}), TSS (14.32 ^{0}Brix) ascorbic acid, (14.78 mg 100 g^{-1}) phenols, (0.49 mg g^{-1}) and B:C ratio (3.99). Significantly higher nitrogen (72.01 kg ha^{-1}), phosphorous (22.71 kg ha^{-1}), potassium (83.04 kg ha^{-1}) and sulphur (14.11 kg ha^{-1}) uptake were recorded in T_7 treatment which was on par with T_4 and T_1 treatments. The soil available nitrogen (189.38 kg ha^{-1}), potassium (391.57 kg ha^{-1}) and sulphur (31.13 mg kg^{-1}) after harvest were recorded higher in T_1 treatment with the application of 100% RDF. Higher soil available phosphorous (117.76 kg ha^{-1}) was recorded in T_9 treatment with the application of 50% RDN:K:S with 150% RDP, high buildup of phosphorous were observed. Thus, the study revealed out that application of 100% RDF (150:75:150:30 kg N: P_2O_5:K_2O and S ha^{-1}) found better for *rabi* grown Small onion.

Studies on Zinc and Boron Fractions in Finger Millet - Maize Cropping Sequence under Long Term Fertilizer Experiment

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The present study investigates distribution of different fractions of zinc and boron in soil and their correlations with various soil physico-chemical properties in finger millet-maize cropping sequence under long term fertilizer experiment (LTFE), which has been in progress since 1986. The experiment consists of twelve treatments laid in RCBD with three replications. Soil samples from two depths (0-20 and 20-40 cm) were collected after the harvest of maize crop during the year 2023. Application of FYM @ 15 t ha^{-1} and lime along with 100% RDF improved soil physico-chemical properties as well as different fractions of zinc (*viz*., water soluble, exchangeable, organically bound, amorphous sesquioxide bound, crystalline sesquioxide bound and residual zinc) and boron (*viz*., readily soluble, specifically adsorbed, oxide bound, organically bound and residual boron) significantly compared to absolute control, imbalanced nutrition (100% N and 100% NP) and 100% NPK alone. The per cent contribution of different zinc and boron fractions to the total zinc and boron in soil follows the order of Res-Zn > AMOX-Zn > Org-Zn > CRYOX-Zn > Ex-Zn > WS-Zn and Res-B > Ox-B > Org-B > Spa-B > Rs-B, respectively. All zinc and boron fractions were more or less positively and significantly correlated to each other. Organic carbon and CEC showed a positive correlation with organically bound zinc and boron fractions. The fractionation of zinc and boron, along with soil properties provides valuable insights for improving nutrient management strategies, including organic and inorganic amendments, thereby enhancing soil fertility and supporting soil health.

Effect of Mineral Enriched Organo-Granules Application on Growth, Yield and Quality of Okra (*Abelmoschus esculentus*)

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THE present study was carried out to evaluate the mineral-enriched organo-granules prepared from cow dung, goat dung, and poultry waste on phosphorus release patterns, growth, yield and quality of okra (*Abelmoschus esculentus*) at College of Agriculture, V.C. Farm, Mandya during summer 2024. The granules were prepared by enriching organic wastes with rock phosphate, gypsum and gum acacia as additive. The incubation study revealed gradual increase in P release up to 60 days and stabilized thereafter with respect to poultry waste granules (100% P), that recorded higher labile phosphorus (Available - 28.64 mg kg^{-1} and water soluble P-25.17 mg kg^{-1}) and moderately labile phosphorus (Saloid bound -28.1 mg kg^{-1} and organic bound P -134.38 mg kg^{-1}) followed by cow dung granules. In contrast, goat dung granules exhibited a slower nutrient release for the first 60 days and increased as the incubation period progressed. In pot experiments, poultry waste granules (100% RDP, T9) significantly enhanced okra's growth and yield parameters *viz.,* plant height (30.21 cm), leaf count (23.33), and fruit yield (466.57 g). This treatment also improved quality attributes like crude fiber (8.21%) and protein content (23.81%) along with higher macro and micro-nutrients (N, P, K, Ca, Mg, S, Fe, Mn, Zn and Cu) uptake by Okra and availability in the post-harvest soil. Cow dung granules (100% RDP, T3) ranked second, while goat dung granules (50% RDP, T8) showed the lowest performance. The results demonstrate that poultry waste-based organo-granules are the most effective in improving okra's growth, yield and soil nutrient status, followed by cow dung granules.

Mapping of Salt Affected Soils and Soil Quality Index in Cauvery Command Area of Karnataka Using Geospatial Techniques and Machine Learning Algorithms

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SOIL quality assessment and mapping of salt-affected soils are critical for sustainable land management in command areas. The study intended to assess soil properties, their spatial variability and soil quality in the Cauvery command area of Mandya district using geospatial technique and machine learning algorithms. Surface soil samples along with ancillary data were collected following randomized survey to ensure spatial representation across different landscapes in the study area. Principal component analysis was used to develop a minimum data set for Soil Quality Index (SQI) estimation, while Digital Soil Mapping (DSM) techniques were employed to optimize soil property predictions. The study found that lowlands exhibited higher soil moisture constants and exchangeable cations due to their finer texture and leaching, while uplands had coarser texture and lower moisture retention. The Random Forest demonstrated superior predictive performance for most of the soil properties compared to Support Vector Regression machine learning algorithm. Further, feature selection had improved prediction accuracy for soil properties. Soil quality mapping at finer spatial resolution (10 m) using non-linear scoring provided the best results ($R^2 = 0.54$), effectively capturing spatial variability. The SQI values ranged from 0.28 to 0.80 with higher values in central and southern regions due to favourable properties like base saturation and texture, classifying 45 per cent of the area under medium quality, 38 per cent as low and 16 per cent as high quality. Predicted maps of salt-affected soils indicated that 65.6% of the area was slightly saline, 34.4% non-saline and minimal area (12 ha) under moderately saline, signalling early salinity stress due to intensive monocropping of sugarcane and paddy. The study highlights the potential of integrating high resolution datasets and machine learning for precise spatial assessment of soil quality and salt affected soils thereby helpful in precision soil management, supporting targeted interventions to enhance soil sustainability in irrigated agricultural landscapes.

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Effect of Finger Millet-Legume Intercropping System on Soil Nitrogen Fractions and Productivity of Crops

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A field experiment was conducted at G-block of Zonal Agricultural Research Station, V.C. Farm, Mandya to evaluate the soil nitrogen fractions in finger millet based legume intercropping system under Southern Dry Zone of Karnataka. The experiment was laid out in a Randomized Complete Block Design with ten treatments and replicated thrice. The treatments consisted of sole crop of finger millet and legume (groundnut, field bean and horsegram) and finger millet & legume intercropping system in 4:2 and 6:2 ratios. The results indicated a marginal increase in soil nitrogen fractions with advancement of crop growth. At harvest the soil nitrogen fractions *viz.*, inorganic fractions (ammonical nitrogen -24.33 mg kg^{-1} and nitrate nitrogen -11.22 mg kg^{-1}), organic fractions (total hydrolysable nitrogen -445.09 mg kg^{-1}) and microbial biomass nitrogen -27.07 mg kg^{-1}) and total N (799.95 mg kg^{-1}) content were higher in finger millet+groundnut in (4:2) cropping system (T_5). The growth and yield parameters *viz*., plant height (103.64 cm), number of tillers per plant (4.80), number of fingers per ear head (7.25), grain yield (3059 kg ha^{-1}) and straw yield (5264 kg ha^{-1}) were higher in sole crop of finger millet (T_7). Further, the same treatment recorded higher macro (N, P, K), secondary (Ca, Mg, S) and micronutrients (Fe, Mn, Zn and Cu) uptake. However, higher finger millet equivalent yield (3692 kg ha^{-1}), land equivalent ratio (1.29), gross return (Rs.118131 ha^{-1}), net return (Rs.62391 ha^{-1}) and B:C ratio (2.41) were higher in finger millet+ groundnut (4:2) -T_5 cropping system as compared to other intercrops indicating as the most suitable intercropping system for Southern Dry Zone of Karnataka.

Morphological and Phylogenetic Analysis of Endemic *Impatiens* of Western Ghats, India

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The genus *Impatiens* (family Balsaminaceae) comprises approximately 1,120 species, with a significant presence in India's Himalayas and Western Ghats. The Western Ghats is a hotspot for endemic *Impatiens* species, valued for their medicinal properties, bio-aesthetic uses and role in ecosystem studies. However, their habitat specificity and anthropogenic threats have placed many species at risk of extinction. This study examined the distribution, discovery trends and IUCN status of *Impatiens* in the Western Ghats, alongside their evolutionary relationships through phylogenetic and morphological analyses. Data on species distribution, morphology, geographical coordinates, discovery year and conservation status were gathered from secondary sources including book, research paper and thesis. Phylogenetic analysis, using the nuclear ITS gene and UPGMA method, indicated that older taxa have wider distributions, while recently evolved species are more localized, particularly in Karnataka. A total of 161 *Impatiens* taxa have been reported from the Western Ghats, predominantly in South Karnataka and North Kerala. The first species was discovered in 1753, with the most recent in 2024. The unsaturated discovery trend suggests many species remain unidentified. Of the recorded taxa, 111 are classified as threatened under IUCN categories. Morphological analysis deviated from established ecological patterns, indicating a need to reassess classification. This study underscores the rich *Impatiens* diversity in the Western Ghats and the urgency of conservation efforts. Advancing species discovery, reassessing taxonomy and implementing conservation strategies are vital for preserving biodiversity in this ecologically significant region.

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Assessment of Floristic Diversity, Distribution and Carbon Stock in the Biodiversity Heritage Site of GKVK

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THE study was carried out in 14 areas of Biodiversity Heritage Site, GKVK during 2023-2024 for assessing floristic diversity, composition and carbon stock by using RS and GIS techniques. Results revealed that the study area exhibited a greater diversity with less evenness in distribution of species. The vegetation structure was very complex with good stand density and basal area with good regeneration status as study area showed reverse 'J' shape pattern (girth class distribution). More than 75 per cent of the carbon was stored by only top 15 dominant trees (based on IVI) in the heritage site. All the soil properties showed significant variation among the areas and across the depths (0-30, 30-60 and 60-90 cm). Area D (Botanical Garden) had more diversified flora and distribution comparatively and E7 (Tree arboretum) recorded highest tree biomass and carbon. While SOC, litter carbon and nutrient turnover were recorded more in area C (Scrub Forest) and litter accumulation was highest in area E6 (Plantation and Orchards). Carbon stock per hectare from AGBC, BGBC, soil C and litter C was also recorded more in Area C indicating that these natural scrub forests are of more ecologically significant as they are capable of sequestering more carbon. Basal area, biomass and carbon were more in lower girth class individuals and kept on decreasing with increasing girth, but in the last girth class (>180 cm) all three parameters increased for the entire study area. A strong relation was observed between AGB and carbon with stem density (R^2=0.806) and also with basal area (R^2=0.614). Geospatial modelling revealed that 68 per cent of accuracy was observed between predicted and estimated biomass and carbon for the entire study area with a mean value of 884.52 kg/100 m^2 and 415.67 kg/100 m^2 for biomass and carbon, respectively.

Studies on Crop-Weather Relationship in Pigeonpea

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PIGEONPEA is an important pulse crop widely cultivated under rainfed conditions in semi-arid regions of southern India, where its productivity is highly influenced by climatic variability and weather extremes. The present investigation was carried out at AICRP on Agrometeorology, University of Agricultural Sciences, Bangalore, during *kharif* 2024-25 to evaluate the effect of weather, sowing time and genotypes on the growth, yield and resource use efficiency in pigeonpea. The study comprised three varieties *viz*., BRG-1, BRG-5 and PRG-176 sown during three dates: May II FN, June I FN and June II FN. Among genotypes, PRG-176 exhibited superior heat, photo-thermal, helio-thermal, radiation and water use efficiencies, recorded higher grain yield (1011 kg ha^{-1}) owing to enhanced reproductive efficiency and resource utilization. Correlation analysis revealed significant influence of rainfall, temperature and radiation on yield during critical phenological stages. Further to project the yield, evaluation of six CMIP6 models revealed the reliability of MIROC-ES2L. Among predictive models, the Artificial Neural Network achieved the higher accuracy ($R^2 > 0.99$) and subsequently used to identify dominant weather predictors and forecast yield for the near (2025–2040), mid (2041–2070) and far future (2071–2100) under SSP2-4.5 and SSP5-8.5 scenarios. Results indicate that BRG-1 (July sowing) and TTB 7 (June sowing) are likely to sustain higher yield and exhibit greater resilience under high-CO_2, wetter conditions projected for SSP5-8.5, making them promising candidates for climate adaptation. Incorporating the genotypic coefficients of these varieties into breeding programmes could further enhance yield stability and support long-term climate-resilient pigeonpea production.

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Development and Performance Evaluation of A Small-Scale Flaking Machine for Ready-To-Cook (RTC) Pulses

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As the demand for ready-to-cook (RTC) and ready-to-eat (RTE) foods continues to rise, the need for cutting-edge processing technologies becomes more critical. This study focuses on the design, development and performance evaluation of a small-scale flaking machine for ready-to-cook (RTC) pulses, aiming to reduce cooking time and increase the consumption of pulses in daily diets by enhancing their convenience and nutritional value. The developed small-scale flaking machine was used to optimize moisture content (%), roller clearance (mm) and operating speed (rpm) for enhanced flake quality and process efficiency, using Response Surface Methodology (RSM). The performance of machine was evaluated with pigeonpea and greengram to assess its efficiency in flake production. The optimized parameters for production of pigeonpea flakes were moisture content of 24.31 % (wb), roller speed of 227 rpm, and roller clearance of 0.44 mm, achieving a flaking efficiency of 88.37 per cent, throughput capacity of 14.20 kg/h, breakage of 8.59 per cent, spillage of 4.10 per cent and power consumption of 0.25 kW. For green gram, the optimized parameters for production of flakes were moisture content of 24.53 % (wb), roller speed of 250 rpm, and roller clearance of 0.35 mm, resulting in a flaking efficiency of 96.35 %, throughput capacity of 15.25 kg/h, breakage of 1.94 per cent, spillage of 2.76 per cent and power consumption of 0.10 kW. The maximum throughput capacity of the developed machine was 23.26 kg/h for pigeon pea and 20.41 kg/h for greengram with peak throughput observed at a roller speed of 350 rpm. The total fabrication cost of the developed flaking machine was estimated at Rs.41,450, with an operating cost of Rs.2.16 per kilogram of processed flakes.

Studies on Shelf-Life Extension of Indian Oil Sardine (*Sardinella longiceps*) by In-Package Cold Plasma Technology

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INDIAN OIL sardine (*Sardinella longiceps*) is highly nutritious and perishable in nature and demands shelf-life extension. Research was conducted using vacuum cold plasma technology, as it is an effective, non-toxic and non-thermal technology. The study aimed to extend the shelf-life of Indian oil sardine using in-package cold plasma (CP) technology and to study its effects on biochemical and microbiological properties of the treated fish. The treatment was performed using three voltages and time durations (30, 40, 50 kV and 3, 4, 5 minutes). CP treatment resulted in an increase in protein content, followed by a minimum decrease during storage. A significant reduction in pH and moisture content was observed after treatment, but both showed an increase during storage. Colour analysis of both L^* and a^* values showed a decreasing trend after CP treatment compared with untreated samples, but they increased during storage. In contrast to L^* and a^*, the b^* value showed an opposite trend in the treated samples. A significant increase in hardness was observed after treatment, although it decreased during storage, while adhesiveness exhibited an opposite trend. A significant increase in TBARS was observed in all treated samples. A significant reduction in microbial load was noted following all treatments. OPSTAT software was used for statistical analysis and T4(40 kV, 3 minutes) was found as the optimized treatment, with a shelf-life extension of 15 days. On the 3rd day of storage, the following values were recorded for the fillets treated under the T4 condition: protein content: 20.13 g/100 g, moisture content: 9.21%, pH: 6.29, L^*: 30.87, a^*: 4.46, b^*: 9.68, hardness: 1419.92 N, adhesiveness: 1.47 N, TBARS: 0.42 mg MDA/kg, LAB: 0.459 log cfu/g, Pseudomonas: 0.23 log cfu/g and Enterobacteriaceae: 0.027 log cfu/g. The B:C ratio for production of 1 kg of VCP treated Indian oil sardine was 1.14:1.

Design and Development of Portable Honey Extractor for Domestic Bees

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Honeybees play a vital role in ecological balance and agricultural productivity through pollination, while honey serves as a natural sweetener with medicinal properties. Conventional manual honey extraction methods often lead to contamination, honeycomb destruction and low recovery rates, whereas mechanical methods, such as hydraulic or screw presses, do not preserve honeycombs. Centrifugal extractors, though efficient and hygienic, are often bulky, expensive and unsuitable for small-scale operations. To address these challenges, two portable centrifugal honey extractors open and closed models were developed, where open model featured rectangle shape SS-304 baskets of 12 mm x 12 mm square mesh, while the closed model incorporated 1.2 mm SS-304 sheet of leak proof baskets with sloped bottoms for efficient honey collection. Both designs included a central vertical shaft, horizontal shaft and bevel gears with a 1:3 ratio for smooth manual operation. Performance evaluations at speeds of 150–350 rpm and extraction duration of 1–3 min revealed that the open model achieved a maximum honey recovery of 95.98 per cent and an extraction rate of 3.44 g/s, while the closed model achieved 97.03 per cent recovery and a 3.11 g/s extraction rate. However, undue vibrations were observed at speeds above 300 rpm. Optimal performance was observed at 250–300 rpm for 2 min. The open model recorded a honey recovery of 92.54 per cent with an extraction rate of 2.55 g/s, and a desirability score of 0.929, while the closed model achieved 94.94 per cent recovery with an extraction a rate of 2.06 g/s, and a desirability score of 0.935. The extracted honey's physicochemical properties met regulatory standards with variations attributed to floral sources and handling practices. The developed extractors are portable, cost-effective, versatile and food-safe. They provide a sustainable solution with reduced drudgery ensuring efficient honey recovery without honeycomb damage.

Evaluation of Tractor Drawn Seed-cum-Fertilizer Drills and Mulching for *in-situ* Soil Moisture Conservation to Enhance the Productivity of Finger Millet

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The study conducted during *kharif* season of 2023 at AICRP for Dryland Agriculture, UAS, GKVK, Bengaluru aimed to evaluate the performance of three tractor-drawn seed-cum-fertilizer drills along with soil moisture conservation through mulching with finger millet straw and dry leaves to enhance soil moisture conservation and crop productivity. The experiment revealed the significant differences in moisture conservation, growth and yield of crop. The UAS-B improved seed-cum-fertilizer drill (S_1) exhibited better performance, with respect to higher draft (2.55 kN), field capacity (0.81 ha/ hr), working speed (1.16 m/s), field efficiency (83.07%), and reduced working time (1.46 h/ha). Plant population recorded highest in S_1 on 8th day after sowing as 170/m^2 as compared to S_2 and S_3 values (68.67/ m^2, 73.12/ m^2). Soil moisture content recorded at 7.5 cm and 15 cm depth was highest in S_1 (9.89 and 13.15%, respectively). Mulching (M_1) also recorded better moisture content (9.80 and 3.34%, respectively). Growth parameters, including plant height, no of tillers, no of leaves per plant, leaf area index, and total dry weight, were significantly higher in S_1 (88.27 cm, 4.72, 30.68, 2.73, 50.62 g) and M_1 (88.48 cm, 4.55, 35.67, 2.75, 48.50 g). Yield parameters showed the highest grain (28.28 q/ha) and straw yield (50.12 q/ha) in S_1, while M_1 recorded 26.37 q/ha grain and 51.36 q/ha straw yield. Economic analysis revealed net returns of Rs.61,200/ha with B:C ratio of 2.58 in S_1 and Rs.35,309/ha with B:C ratio of 2.16 in M_1.The research reveals that the effective use of the UASB improved seed-cum-fertilizer drill helps to compact the soil, provide proper sowing of seeds and fertilizer with a thick soil cover. This enhances moisture conservation in the soil. Additionally, the practice of mulching reduces moisture evaporation and further improves soil moisture retention.

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