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SCOPE OF COMMERCIAL DAIRY FARMING IN INDIA - A REVIEW

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Abstract

India's commercial dairy farming sector has emerged as a pivotal component of the nation's agrarian landscape, contributing significantly to the economy and the livelihoods of millions of farmers. With a rich history rooted in traditional practices, dairy farming has evolved to meet the demands of a growing population and changing consumer preferences. The total livestock population in India is estimated at 535.78 million as of the 21st Livestock Census in 2024. This census, conducted from October 2024 to February 2025, counted 15 species of domesticated animals, including cattle, buffalo, goats, and sheep. The total population reflects a 4.6% increase from the 20th Livestock Census in 2012. As India stands as one of the largest producers of milk globally, the potential for commercial dairy farming is immense, driven by increasing urbanization, rising incomes, and a burgeoning middle class that seeks quality dairy products. However, the sector is not without its challenges, including infrastructure deficiencies, financial constraints, and health-related issues among livestock. This article reviews the scope of commercial dairy farming in India, exploring current trends, technological advancements, government initiatives, and future opportunities while also addressing the sustainability of and challenges faced by dairy farmers in this dynamic industry.

Introduction

Historical Background: Dairy farming has an ancient history, dating back to the domestication of animals for milk production, with evidence of cheese production and milk usage as early as 7,000 BC. Goats were likely the first animals to be milked, and by the 3rd millennium BC, people began processing milk beyond simple raw consumption. India boasts a large and diverse livestock population. The 20th Livestock Census in 2019 reported the total livestock population to be 535.78 million, an increase of 4.6% compared to the previous census in 2012. The total bovine population, which includes cattle, buffaloes, mithun, and yaks, reached 302.79 million. Over time, dairy farming has evolved with significant advancements in milk production and safety, and modern practices like pasteurization emerged in the early 1900s. Historically, dairy farming was a small-scale activity, often confined to households.

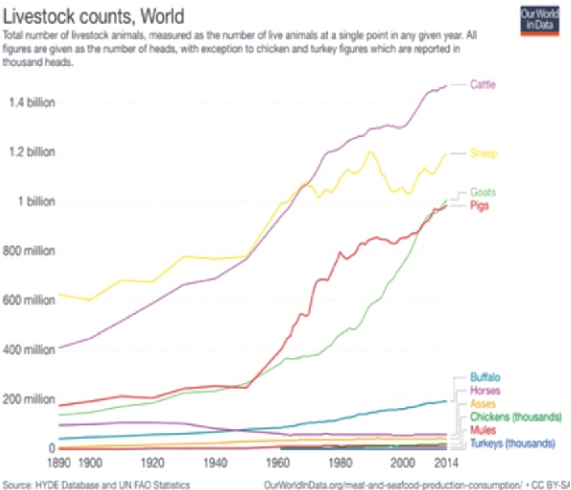
However, with the Green Revolution and subsequent policy initiatives like Operation Flood in the 1970s, commercial dairy farming flourished. This program transformed India into the world's largest milk producer, with various regional cooperatives leading the effort. Today, dairy has transcended mere tradition and has become a major player in the agricultural landscape. **Significance of Dairy Farming in the Indian Economy:** Dairy farming is more than just milk and butter—it's the backbone of rural livelihoods in India. Contributing approximately 4.6% to the national GDP, it engages millions of farmers, especially women, who often manage the milking and production processes. It serves as a source of steady income for families and provides essential nutrients through milk and its products. Plus, let's not forget about that lovely cup of chai made from fresh milk! So, whether you're a farmer, a consumer, or someone who just enjoys the

occasional dairy-filled dessert, dairy farming plays a vital role in India’s economic landscape.

Current Trends and Market Dynamics:

- **Consumption & Production Statistics:**
Global output: World milk production (cow, buffalo, sheep, goats) was about 940–950 MMT in 2022–23 (FAO/OECD estimate) and is growing ~1–2% per year overall. IFCN reports ~539,650 thousand tonnes of cow-milk (cows only) in 2020, rising to ~558,475 by 2025.
- **Major producers:** India is by far the largest, followed by the EU, the USA and China. India’s cow and buffalo milk was ~93.8 MMT in 2020 (cows only 93.8 MMT, plus buffalo), rising to ~103.2 MMT in 2025. The EU-27 (as one region) produced ~145 MMT in 2024, about the same as in 2020. The USA produced ~102–103 MMT annually. China (mostly cow’s milk) was ~43 MMT in 2024 and growing slowly.
- **Domestic consumption:** Most milk is consumed domestically. India’s consumption is ~216 MMT (cow+buffalo) in 2024. Its fluid milk consumption is forecast to be ~91 MMT in 2025. In contrast, EU fluid milk use is small (only 23.6 MMT in 2024) because most milk there goes into cheese and powder. The US uses ~102 MMT fluid (2025).
- **Per-capita patterns:** Per-person milk intake is highest (50+ kg/yr) in Europe, the Americas and some Central Asian countries, but only ~60+ kg in India (which, despite being the largest aggregate consumer, has ~400 kg/yr total for 1.4 billion people). Growing per-capita intake in developing regions is key to demand growth.
- **Policy, Trade and Economic Factors:** Many policy and economic factors shape dairy demand. India remains highly protective of dairy: it applies steep import tariffs (30–60% on dairy) to support local farmers. The government also runs major dairy development programs (e.g. National Programme for Dairy Development) with large budgets. These policies, along with rising domestic demand, mean India

imports almost no milk but is the world’s top producer. Trade talks (e.g. US-India negotiations) have so far left dairy largely closed to imports.



In the EU, policies aim to balance production with environmental goals. New green regulations and subsidy reforms have kept EU milk output flat, while premium prices for cheese and butter encourage the processing of available milk into value-added products. China has also shifted to self-sufficiency: it grew dairy output and stockpiled powders so it could cut imports (its cheese imports are rising, but it even reduced milk and WMP imports in 2023). In contrast, Argentina illustrates how macro shocks can swing dairy sharply: an economic crisis in 2023 caused a 13% drop in milk output (H1 2024), which sent global prices higher and boosted its exports on a weaker peso.

Market Size and Growth Rate:

Global dairy demand has rebounded strongly in 2023–24. According to IFCN data, global milk production grew ~2.2% in 2023, and total dairy demand rose ~2.4%, partly reversing 2022’s sluggishness.

Summary Table: Market Growth by Region and Segment			
Market / Segment	2023/24 Value	CAGR (mid-term)	Notes
Global Dairy (total)	~\$881B	~5.2% (to 2030)	Driven by the Asia-Pacific, high-protein trend
India Dairy Market	\$135.3B (2024)	~9.3% (2024–32)	~25% of world production; strong policy support

Global Plant-Dairy	\$32.4B (2024)	~13.9% (2024–32)	Fastest growth, but still <5% of total dairy
Global Yogurt	\$142B	~5–6% (APAC)	APAC ~53% of sales; India +8.7% CAGR
North America Dairy	~ \$480B*	~3–4%*	Mature market, organic and protein segments rising
Europe (EU) Dairy	~ \$150B*	~1–2%*	Stable overall; cheese demand is strong
Asia-Pacific Dairy	~ \$500B*	~6–7%*	High growth (China, SE Asia); India largest producer

*Estimates from industry reports. Growth for NA/EU/AP is not directly cited but is indicative of market sentiment.

Developing countries drove most growth: China, India, and Southeast Asia expanded consumption, while exports from major suppliers fell as top importers like China focused on domestic supply. Industry forecasts expect this momentum to continue: one analysis projects global milk consumption rising by 113 million tonnes (about +11%) by 2030, driven by population and income gains in developing regions. In dollar terms, the global dairy products market was roughly \$881 billion in 2023, with a projected ~5.2% annual growth through 2030. Overall, strong demand in Asia (especially South Asia and China) is balancing mature or flat demand in Europe and North America.

Key growth drivers include population and income growth, particularly in Asia and Africa, which lift overall dairy consumption. Rising disposable incomes and urbanization spur demand for convenience and processed dairy (milk, packaged yogurt, cheese, etc.). Nutrition and health trends also matter: dairy's protein content and perceived health benefits (especially of yogurt and cheese) are attracting consumers. In India specifically, for example, rising income, a large youth population, expanding supermarket/e-commerce channels, and aggressive marketing have lifted both fluid milk and value-added dairy demand. Government support and programs further boost production and supply; the USDA notes India's milk output is rising partly due to "continued government support for the development of the national dairy sector" (breeding, price incentives, etc.).

Developed and developing markets show different patterns. Developed markets (US, EU, etc.) have high per-capita dairy intakes but slower growth. In the US and EU, fluid milk consumption is mostly flat or gently declining on a per-capita basis, although total consumption may tick up with population. Instead, growth in these markets is concentrated in niche segments (e.g. organic, high-protein products) and processed dairy. For instance, US fluid milk sales actually rebounded in 2024: whole milk demand rose ~3.2% and total milk sales grew ~2%, as some consumers shifted back from pricier plant milks. Likewise, Americans' cheese consumption is at record highs (up ~20% over ten years), and butter and specialty cheeses are enjoying steady demand. In contrast, developing markets (like India, China, Latin America, Africa) are seeing brisk growth from a low base. India's per-capita consumption remains well below international benchmarks, but it is rising year-over-year. For example, India's total fluid milk consumption is forecast to grow from 89 MMT (2024) to 91 MMT (2025), driven by population growth and income. China's dairy intake is also expanding as more urban and affluent households add milk, yoghurt and cheese to their diets. In short, high-income markets focus on premium, value-added or speciality dairy, whereas low-/middle-income markets are still expanding basic dairy consumption. (One expert notes global milk demand growth is currently ~0.9% due to population and ~1.4% due to rising per-capita intake, reflecting both demographic and diet changes.)

The Indian dairy market is bigger than your uncle's BBQ grill at a family reunion! With an estimated market size of over \$140 billion and an annual growth rate hovering around 15-20%, the sector is booming. This growth is propelled by urbanization, increasing disposable incomes, and changing consumption patterns. In a nutshell, more people want that creamy goodness, and dairy farmers are answering the call—one cow at a time!

- **Consumer Preferences and Changing Dietary Patterns;** Let's face it: Gone are the days when milk was just for children or coffee. The modern-day consumer is all about options. From almond milk lattes to probiotic yoghurt, preferences have diversified

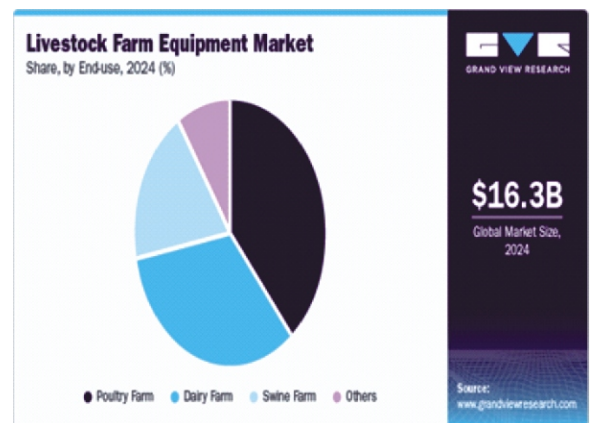
dramatically. Health-conscious millennials are driving demand for organic and fortified dairy products. If it doesn't come with a hint of super food or a catchy Instagram hashtag, they're likely to swipe left.

- **Regional Variations in Dairy Production:** flavours of ice cream, dairy production varies widely across regions in India. States like Uttar Pradesh, Rajasthan, and Gujarat lead in milk production, thanks to favourable climatic conditions and strong cooperative networks. While some areas thrive on cow milk, others are synonymous with buffalo milk—each with its unique flavour profiles and cultural significance. So whether you're from the North, South, East, or West, you'll find your local dairy delight!
- **Key Challenges Facing Dairy Farmers:** the major challenges are disease outbreaks (e.g., FMD), lack of skilled manpower, Feed and fodder availability, Cold storage and logistics, and Climate change impacts.
- **Infrastructure and Supply Chain Issues:** infrastructure—the Achilles' heel of many an ambitious dairy farmer. Poor transportation networks, inadequate cold storage facilities, and a lack of access to modern technology can turn fresh milk into sour disappointment. With a product as perishable as milk, every moment counts! It's like trying to make a soufflé with a faulty oven; one wrong move, and it all falls flat.
- **Financial Constraints and Access to Credit:** While dairy farming has the potential to be lucrative, financial hurdles often loom large. With limited access to credit facilities, many farmers struggle to invest in modern equipment or quality feed. It's a bit like trying to run a marathon in flip-flops—possible, but not easy! The government and various organizations are stepping in to try and bridge this gap, but progress can be slow.
- **Health and Breeding Challenges:** In the land of diverse breeds and dairy products, ensuring the health of cattle remains a primary concern. Issues such as diseases, poor breeding practices, and nutritional deficiencies can severely impact milk yield

and quality. Not to mention, farmers often wrestle with the balance between traditional practices and modern innovations. It's a delicate dance; one misstep can lead to serious consequences for both the cows and the farmers.

Technological Advancements in Dairy Farming

- **Innovations in Breeding Techniques:** If you've ever thought about playing matchmaker, welcome to the world of dairy farming! Innovations in breeding techniques, like artificial insemination and genomic selection, are helping farmers produce healthier and more productive animals. The result? Cows that are not just milking machines, but also the crème de la crème of bovine genetics. It's a whole new level of Moo-deration!



- **Automation and Smart Farming Solutions:** Say goodbye to the days of hand-milking and hello to automation! With dairy farms adopting smart farming solutions, tasks ranging from milking to feeding can now be handled by machines. Drones, sensors, and robotic milking machines are making farming more efficient. Who knew that farming could involve so much tech?
- **Data Analytics and Farm Management Software:** In today's data-driven world, even cows have their own management software! Farmers are using data analytics to track milk yield, animal health, and feed efficiency. Farm management software allows for real-time monitoring and decision-making, helping farmers become more productive. So, if anyone ever tells you

farming is just about dirt and cows, remind them it's also about data and tech—because what's a cow without a bit of code? And there you have it! The world of commercial dairy farming in India is vibrant, complex, and full of potential, just like the perfect glass of milk.

Government Policies and Support Initiatives

- **Subsidies and Financial Assistance Programs:** When it comes to commercial dairy farming in India, the government knows how to butter the bread, especially for aspiring dairy entrepreneurs! Through various subsidies and financial assistance programs, the government provides a cushion for farmers like; Rashtriya Gokul Mission, National Dairy Plan (NDP), Dairy Entrepreneurship Development Scheme (DEDS), Subsidies on; Dairy infrastructure, Cattle feed, Veterinary services, Easy availability of loans and grants via NABARD and banks for dairy setup. These initiatives aim to lower the cost of dairy production and encourage investments in modern technologies. From grants for setting up dairy units to interest subsidies on loans for purchasing high-breed cattle, these financial supports are as essential as that morning cup of cha for many dairy farmers.
- **Regulatory Framework and Quality Standards:** Navigating the sea of regulations can feel like wandering through a dairy aisle blindfolded. But fear not! India's regulatory framework concerning dairy farming is designed to ensure that consumers get quality products while farmers adhere to food safety standards. The Food Safety and Standards Authority of India (FSSAI) keeps a watchful eye, ensuring that hygiene and quality are never compromised. This not only helps in building consumer confidence but also opens doors for dairy products in the international market. So, while the paperwork may seem daunting, it's all aimed at promoting a wholesome industry.
- **Training and Development for Farmers:** You wouldn't trust just anyone to make your favorite latte, right? Similarly, dairy farmers need proper training to ensure they're

equipped with the best practices and innovations. Various government and non-government organizations conduct training programs that cover everything from artificial insemination techniques to the use of technology in dairy farming. These initiatives are crucial for skill enhancement, ensuring that farmers are not only milking cows but also maximizing their potential profit, like turning milk into gold!

Sustainability and Environmental Considerations

- **Water and Land Resource Management:** In a country where monsoons can be more unpredictable than your favourite reality show, managing water resources is critical for dairy farming. Sustainable practices like rainwater harvesting and efficient irrigation techniques help conserve water. Additionally, land resource management through crop rotation and sustainable feed practices ensures that the nutrients in the soil are preserved for years to come. After all, a happy cow needs a happy home!
- **Waste Management and Pollution Control:** Dairy farming is not all about frolicking cows and lush green pastures; there's also the not-so-fun task of managing waste. However, with proper waste management and pollution control measures, dairy farmers can turn what was once a headache into a resource. Technologies like bio-digesters convert waste into biogas, allowing farmers to power their operations sustainably while significantly reducing their carbon footprint.
- **Organic Dairy Farming Practices:** Organic dairy farming is the new black, and for good reason. With a growing number of health-conscious consumers, the demand for organic milk and dairy products is skyrocketing. By employing practices free of synthetic fertilizers and pesticides, farmers can tap into this lucrative market while promoting biodiversity and environmental health. So, whether it's organic ghee or curd, consumers can feel good about their choices, and farmers can enjoy a premium price tag!

Future Prospects and Opportunities:

- **Export Potential and Global Market Trends:** India's dairy sector is poised for a global milkshake party, and it wants to be the guest of honour! With a rich variety of dairy products and increasing international demand, India has immense export potential. The country's unique offerings, such as traditional Indian cheeses and yoghurts, are catching the eyes of global consumers. With strategic investments and better marketing, the Indian dairy sector could soon be milking its way into markets around the world.
- **Emerging Consumer Trends:** Health and Wellness. As consumers become increasingly health-conscious, the demand for nutritious dairy products is on the rise. Fortified milk, probiotic yoghurts, and low-fat options are just a few of the trends shaking things up in the market. By aligning with these trends, dairy farmers can not only cater to health enthusiasts but also ensure steady revenue streams. So, let's raise a glass of calcium-packed goodness to health and wellness!
- **Collaboration with Startups and Innovation Hubs:** In this digital age, collaboration is the name of the game. Dairy farmers have much to gain from partnering with startups and innovation hubs that focus on agri-tech solutions.

Conclusion

The path forward for dairy farming in India is that the country has a vast domestic market, Government backing, advancing technologies, and emerging export channels. With the proper planning, training, and investment, it's a high-potential agri-business sector.

- **Strategic Recommendations for Stakeholders:** For dairy farming in India to truly thrive, a collective effort is needed. Stakeholders must focus on forming cooperative societies, improving infrastructure, and Growth in Online milk delivery platforms, Cold-chain logistics, Export of dairy products, Private sector

investments in dairy processing units and packaging and investing in research and development. By embracing technology and sustainable practices, farmers can cultivate not just crops but a prosperous future for the industry. Let's ensure that dairy farming goes from strength to strength—without getting bogged down in bureaucracy!

- **Vision for the Future of Dairy Farming:** The future of dairy farming in India looks bright, but it requires vision and adaptability in the face of changing consumer demand and environmental challenges. A commitment to innovation, sustainability, and quality will help propel the sector forward, ensuring that it remains not just viable but vibrant. So, here's to a robust dairy farming landscape, one that's rich in opportunities and ready to moo-ve into the future! In conclusion, the scope of commercial dairy farming in India presents both significant opportunities and formidable challenges. As the sector continues to evolve in response to market demands and technological advancements, it is crucial for stakeholders, ranging from farmers to policymakers, to collaborate in overcoming obstacles and enhancing productivity. By embracing innovative practices and sustainable approaches, the dairy industry can not only secure its place as a vital economic driver but also contribute positively to the livelihoods of millions. With the right support and strategic vision, the future of commercial dairy farming in India holds great promise for growth and development.

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CORRELATION ANALYSIS UNDER CHARCOAL ROT DISEASE

STRESS CONDITIONS IN MAIZE (*ZEAMAYS* L.)

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Abstract

Maize (*Zea mays* L.), the “Queen of cereals,” ranks third globally in production after wheat and rice and is a major food, feed, and industrial crop. The study evaluated 16 diverse maize lines at ICAR-IIMR, Ludhiana, and multi-location sites (New Delhi and Dharwad) to assess associations among yield and its contributing traits. Plant architecture, maturity, flowering, and yield-related characteristics were all documented. Plant height shown significant positive correlations with ear parameters and yield, while days to anthesis and silking were strongly linked ($r_p = 0.964^{**}$, $r_g = 0.752^{**}$) and closely associated with days to maturity. Ear length and diameter were strongly related to kernel number per row and grain weight, whereas field weight and grain weight were strongly correlated ($r_p = 0.979^{**}$, $r_g = 0.749^{**}$), indicating field weight as a good yield estimator. Strong genetic relationships between plant height, kernel rows, kernel number, and field weight indicate indirect selection for increasing charcoal-rot resistance and grain yield under stress, but phenotypic correlations were greater than genotypic ones.

Introduction

Maize, known as Queen of cereals, is the third most important cereal crop in the world ranks just after wheat and rice. Respect to the global area under cultivation, the maize crop has a cultivated area near about 180 million hectares (M ha) globally that contributes approx. 50% (1,117 million metric tons) to the global grain production (Prasanna, 2018). Besides being used as foods, maize is also being used as raw material for several industries like food processing and feed industries (Prasanna, 2018) and production of biofuels. A rapid progress in maize in terms of both production and productivity has been observed among different Asian and South Asian countries

that ultimately holds about 31% share in global maize production (Zaidi *et al.*, 2018). The correlation analysis measures the associations between two or more than two variables. Correlation of high value between various characters indicates the degree to which different characters of a plant are associated with economic productivity (Ahsan *et al.*, 2008). The directly observed association between two characters is phenotypic correlation, while genotypic correlation expresses the extent to which two traits are genetically associated. Both genotypic and phenotypic correlations among and between pairs of agronomic traits provide scope for indirect selection in a crop breeding program.

Material and Methods

The current research, was conducted at the ICAR-Indian Institute of Maize Research, Ludhiana, where the experiment was carried out. Additionally, multilocation trials were conducted at three locations, including ICAR-IIMR, Ludhiana; ICAR-IIMR, New Delhi; and University of Agricultural Sciences Dharwad during Spring 2022 and Rabi 2021–22 to identify the relationship between yield and its contributing traits of 16 diverse lines including hybrids, parental lines, backcrosses and F₂ populations.

Location of the experiment and time period

The experimental investigation was carried out at the ICAR-IIMR, Ludhiana; ICAR-IIMR, New Delhi; and University of Agricultural Sciences Dharwad during Spring 2022 and Rabi 2021–22.

The experimental design was structured to maximize the precision of relationship between yield and its contributing traits across the locations. To ensure uniform growth and accurate data collection, the planting layout was meticulously planned. Plants were spaced at 20 cm between individuals and 60 cm between rows. Each row was 4 meters long, providing sufficient space for plant development and disease expression.

To achieve a comprehensive understanding of the association between different yield and its attributing traits under diseased condition, the study was conducted using correlation analysis. The experimental setup was carefully designed to ensure comprehensive data collection and to accurately represent the genetic variability present in the maize populations. The following yield contributing traits are being evaluated for correlation analysis with yield. Plant Height (cm), Days to 50% Tasseling, Days to 50% Silking, Days to Maturity, Ear Height (cm), Ear Length (cm), Ear Diameter (cm), Number of Kernel Rows per Ear, Number of Kernels per Row, 100-Kernel Weight (g), Grain Yield per Plant (g), Yield per Plot (g).

For evaluating the performance of different maize accessions in terms of agronomic traits, these parameters were carefully measured and

recorded. The collected data provided a comprehensive understanding of how various traits contribute to overall yield and plant performance. This approach enabled the identification of promising lines for future breeding programs aimed at improving maize productivity.

Artificial Media Preparation for *Macrophomina phaseolina*

The process involves the isolation and preservation of *M. phaseolina* for inoculation and screening purposes. The pathogen is commonly grown on Potato Dextrose Agar (PDA), which contains 200 g of potato, 20 g of dextrose, 15 g of agar, and 1 litre of distilled water.

The preparation involves boiling potato pieces in water, filtering the extract followed by adding dextrose and agar. The pH is adjusted to 5.6–6.0, and the medium is autoclaved at 121°C for 15–20 minutes. Small pieces (2–5 mm) of diseased maize leaf samples inoculated onto PDA plates under aseptic conditions. The plates are incubated at 28–30°C for 5–7 days in an incubator.

Protocol for Disease Inoculation

The objective of this procedure is to inoculate maize plants with *Macrophomina phaseolina* to study infection and evaluate host resistance. The inoculum is prepared by culturing the pathogen on PDA for 5–7 days. A suspension of spores or sclerotia is obtained by adding sterile distilled water to the culture plates and gently scraping the fungal growth. The suspension is adjusted to a concentration of approximately 10⁶ sclerotia/mL.

Several inoculation techniques are employed:

- **Soil inoculation:** The fungal inoculum (powdered infected sorghum seeds or colonized PDA) is mixed into the soil near the base of maize plants, and adequate soil moisture is maintained to facilitate infection.
- **Stem inoculation:** Small incisions are made on the maize stem using a sterilized needle or scalpel. A PDA disc containing *M. phaseolina* is placed on the incision and secured with parafilm or tape.
- **Seedling inoculation:** Maize seeds are coated with fungal spores or sclerotia before sowing. Seedlings are then grown under controlled conditions for 7–14 days.

These methods help ensure successful infection and enable the assessment of host resistance.

STATISTICAL ANALYSIS

Correlation Coefficient

Pearson correlation coefficient given by Karl Pearson in the 1880s. The solutions are

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}}$$

Where

r = Pearson correlation coefficient

x_i, y_i = Individual sample point

$\bar{x}, \bar{y}$ = Mean of variables X and Y

Summation over all observations

Simple correlation is of three types –

i) Phenotypic ii) Genotypic and iii) Environmental Correlation co-efficient is tested by the following formula with $(n - 2)$ degrees of freedom at 5% and 1% level of significance.

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

Where,

r = Correlation coefficient

n = Number of treatments

The significance of correlation co-efficient (r) was tested by comparing the observed value or correlation coefficient with the tabulated value for $(n-2)$ degree of freedom (Snedecor and Cochran, 1967). If the observed value of 't' is more than the table value of 't' the correlation coefficient is said to be significant.

Result

Table 1: Correlation analysis for yield contributing traits under artificially inoculated condition at Dharwad, Delhi and Ludhiana

Trait	Correlation	DA	DS	ASI	DM	PH	EH	EL	ED	NR	NK	100 GW	FW	GW
DA	rp	1	0.964**	0.06	0.949**	0.388**	0.305**	0.217*	0.063	0.104	0.108	-0.097	-0.13	-0.023
	rg	1	0.752**	0.01	0.850**	0.111**	0.190**	0.130*	0.032	0.086	0.99	-0.085	-0.08	-0.014
DS	rp		1	0.136	0.957**	0.401**	0.315**	0.226*	0.067	0.113	0.116	-0.087	-0.118	-0.033
	rg		1	0.037	0.67**	0.127**	0.250**	0.114*	0.038	0.102	0.101	-0.076	-0.99	-0.021
ASI	rp			1	0.141	0.098	0.067	0.045	0.012	0.021	0.024	-0.012	0.019	-0.044
	rg			1	0.095	0.054	0.047	0.021	0.009	0.011	0.016	-0.004	0.007	-0.021
DM	rp				1	0.421**	0.332**	0.239*	0.071	0.118	0.121	-0.075	-0.105	0.141

Phenotypic Correlation (rp)

It is observable correlation between two variables. It includes both genotypes and environmental effects. Phenotypic correlation is estimated as

$$r_p = \frac{P \text{ Cov}(x, y)}{\sqrt{PV(x) PV(y)}}$$

Where,

rp = Phenotypic correlation co-efficient

P Cov (x,y) = Phenotypic covariance between variable x and y

PV (x) = Phenotypic variance for the variable x

PV (y) = Phenotypic variance for the variable y

Genotypic Correlation (rg)

Genotypic correlation is the inherent association between two characters; it may be either due to pleiotropic action of genes, linkage or more likely to be both. Genotypic correlation co-efficient is estimated according to the following formula:

$$r_g = \frac{G \text{ Cov}(x, y)}{\sqrt{GV(x) GV(y)}}$$

Where,

rg = Genotypic correlation coefficient

G Cov (x,y) = Genotypic covariance between variable x and y

GV (x) = Genotypic variance for the variable x

GV (y) = Genotypic variance for the variable y

Sl.No.	Value of Correlation Coefficients	Rate or Scale
1	>0.65	Very strong
2	0.50 to 0.64	Moderately strong
3	0.30 to 0.49	Moderately weak
4	<0.30	Very weak

	rg				1	0.224**	0.274**	0.114*	0.052	0.104	0.119	-0.065	-0.805	0.101
PH	rp					1	0.801**	0.623**	0.402**	0.512*	0.498**	0.302**	0.421**	0.449**
	rg					1	0.504**	0.401**	0.282**	0.451**	0.390**	0.265**	0.345**	0.325**
EH	rp						1	0.598**	0.375**	0.476**	0.462**	0.281**	0.398**	0.122
	rg						1	0.490**	0.265**	0.442**	0.362**	0.216**	0.297**	0.102
EL	rp							1	0.521**	0.589**	0.572**	0.341**	0.356**	0.354**
	rg							1	0.366**	0.462**	0.361**	0.241**	0.324**	0.250**
ED	rp								1	0.612**	0.598**	0.367**	0.421**	0.555**
	rg								1	0.456**	0.460**	0.241**	0.347**	0.205**
NR	rp									1	0.821**	0.442**	0.489**	0.480**
	rg									1	0.635**	0.350**	0.441**	0.220**
NK	rp										1	0.431**	0.472**	0.463**
	rg										1	0.397**	0.387**	0.224**
100 GW	rp											1	0.512**	-0.009
	rg												1	0.399**
FW	rp													1
	rg													
GW	rp													
	rg													

rg: Genotypic correlation, rp: phenotypic correlation, *Significant at 5% level ** Significant at 1% level DA- Days to anthesis, DS-Days to silking, ASI-Anthesis-silking interval (days), DM-Days to maturity, PH-Plant height (cm), EH-Ear height (cm), EL-Ear length (cm), ED-Ear diameter (cm), NR-Number of kernels rows per ear, NK-Number of kernel rows per ear, 100 GW- 100 Grain weight(g), FW- Field Weight (q/ha), GW-Grain Weight (q/ha).

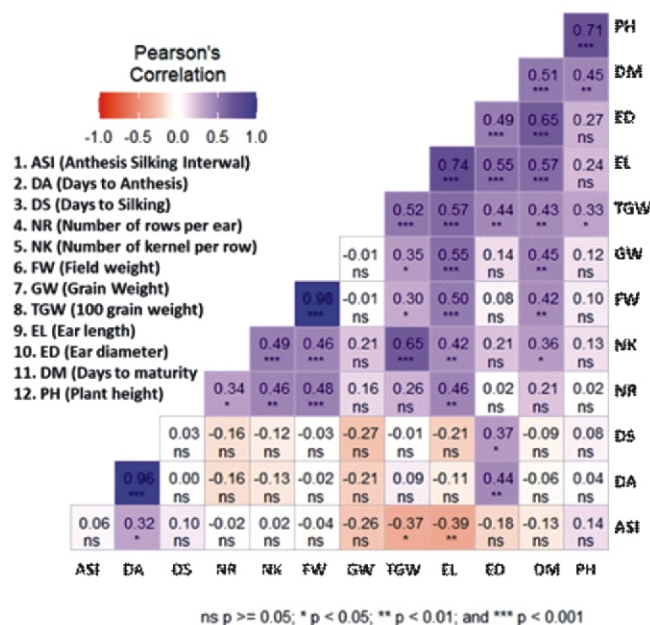


Figure 1: Correlation analysis for yield contributing traits under artificially inoculated condition at Dharwad, Delhi and Ludhiana

DA exhibited very strong positive phenotypic correlations with days to silking (DS; $r_p = 0.964^{**}$)

and days to maturity (DM; $r_p = 0.949^{**}$), alongside moderate associations with plant height (PH; $r_p = 0.388^{**}$) and ear height (EH; $r_p = 0.305^{**}$) table 2. Genotypic correlations mirrored this pattern but were slightly attenuated (r_g DS = 0.752^{**} , r_g DM = 0.850^{**}), with weaker links to yield traits like fresh weight (FW; $r_p = -0.130$) and a minor negative trend with 100-grain weight (100GW; $r_p = -0.097$). These indicate DA as a primary phenological driver, genetically coupled to reproductive and vegetative duration. DS showed near-identical patterns to DA, with $r_p = 0.957^{**}$ to DM, $r_p = 0.401^{**}$ to PH, and $r_p = 0.315^{**}$ to EH, alongside negligible yield impacts (e.g., r_p 100GW = -0.087). Genotypic r_g to DM was 0.670^{**} , underscoring shared QTL for female flowering and maturity timing, critical for pollination synchrony in maize. ASI displayed predominantly weak, non-significant correlations (e.g., r_p DA = 0.06 , r_p DM = 0.141 , r_p PH = 0.098), with r_g values similarly low (< 0.10). This independence suggests ASI as a stress-responsive trait, decoupled from broader phenology and yield architecture. DM correlated strongly with DA ($r_p = 0.949^{**}$) and DS ($r_p = 0.957^{**}$), moderately with PH ($r_p = 0.421^{**}$)

and EH ($r_p = 0.332^{**}$), but weakly with yield (e.g., r_p GW = 0.141). Genotypic r_g PH = 0.224^{**} highlights heritable vegetative-reproductive linkage, influencing harvest index. PH demonstrated the broadest connectivity, with strong r_p to EH (0.801^{**}), EL (0.623^{**}), ED (0.402^{**}), NR (0.512^{**}), NK (0.498^{**}), and yield traits (100GW r_p = 0.302^{**}, FW = 0.421^{**}, GW = 0.449^{**}). Genotypic r_g EH = 0.504^{**} and r_g NR = 0.451^{**} affirm PH as a key architectural hub, often pleiotropically linked to biomass and sink capacity. EH aligned closely with PH (r_p = 0.801^{**}), EL (r_p = 0.598^{**}), ED (r_p = 0.375^{**}), NR (r_p = 0.476^{**}), and NK (r_p = 0.462^{**}), with moderate yield ties (FW r_p = 0.398^{**}). r_g PH = 0.504^{**} indicates robust genetic basis, relevant for machinery harvest ability. EL showed strong r_p to ED (0.521^{**}), NR (0.589^{**}), NK (0.572^{**}), and GW (0.354^{**}), plus PH (0.623^{**}) and EH (0.598^{**}). r_g NR = 0.462^{**} positions EL as a prolificacy enhancer via kernel accommodation. ED correlated positively with NR (r_p = 0.612^{**}), NK (0.598^{**}), FW (0.421^{**}), and GW (0.555^{**}), alongside PH/EH ($r_p > 0.37^{**}$). r_g NR = 0.456^{**} suggests girth contributes to row capacity and yield potential. NR linked strongly to NK (r_p = 0.821^{**}), 100GW (r_p = 0.442^{**}), FW (0.489^{**}), GW (0.480^{**}), and ear traits (e.g., ED r_p = 0.612^{**}). r_g NK = 0.635^{**} underscores its pivotal role in kernel set genetics. NK exhibited r_p = 0.821^{**} to NR, r_p = 0.431^{**} to 100GW, r_p = 0.472^{**} to FW, and r_p = 0.463^{**} to GW, with ear architecture ties (e.g., EL r_p = 0.572^{**}). r_g 100GW = 0.397^{**} reflects sink strength determination. 100GW correlated moderately with FW (r_p = 0.512^{**}) and GW (r_p = -0.009, non-significant), plus NR/NK ($r_p > 0.43^{**}$). r_g FW = 0.399^{**} indicates partial independence from kernel number. W showed very strong r_p to GW (0.979^{**}) and moderate ties to PH (0.421^{**}), NR (0.489^{**}), NK (0.472^{**}), and 100GW (0.512^{**}). r_g GW = 0.749^{**} confirms it as a high-heritability yield surrogate. GW culminated yield correlations, with r_p FW = 0.979^{**}, NR = 0.480^{**}, NK = 0.463^{**}, ED = 0.555^{**}, and PH = 0.449^{**}. Its central position validates comprehensive trait integration for selection.

The heat map also indicates strongest positive association was observed between days to anthesis (DA) and days to silking (DS) with a very high correlation of $r=0.96$ (***) figure 1. Plant height

(PH) showed a strong positive correlation with days to maturity (DM) ($r=0.71$, ***), indicating taller plants tended to mature later. Ear length (EL) had a strong positive association with number of rows per ear (NR) ($r=0.74$,), and also with DM ($r=0.65$, *). Grain weight (GW) was positively correlated with kernel number per row (NK) ($r=0.55$,) and with ear diameter (ED) ($r=0.45$,). Field weight (FW) showed a very strong positive correlation with grain weight (GW) ($r=0.98$, ***), suggesting a close relationship between these two yield traits.

Discussion

Correlation analysis reveals robust associations among key maize traits, aiding breeders in variety selection. Days to anthesis (DA) and days to silking (DS) show near perfect alignment, ensuring synchronized flowering for optimal pollination (Silva *et al.*, 2022). Both traits correlate strongly with days to maturity (DM), indicating that earlier flowering generally accelerates maturity, although minor negative associations exist with grain size (Kakraliya *et al.*, 2021; Oniani *et al.*, 2017). Plant height (PH) exhibits strong ties to ear characteristics, including ear height (EH) and ear length (EL) as well as yield components such as kernel rows per ear (NR), consistent with reports that taller plants support larger ears and higher yields (Vaezi *et al.*, 2000; Kakraliya *et al.*, 2021). Ear length (EL), ear diameter (ED), work together to increase kernel number (NK), which is widely recognized as the primary driver of grain weight (Purna *et al.*, 2024; Aruna *et al.*, 2020). Fresh/Field weight (FW) and grain weight (GW) are closely aligned, supporting the use of FW as a reliable proxy for identifying high yielding genotypes under field conditions (Purna *et al.*, 2024; Silva *et al.*, 2022). Phenotypic correlations are generally higher than genetic correlations, confirming that environmental factors shape trait associations, even though genetic correlations still provide a sound basis for breeding decisions (Vaezi *et al.*, 2000; Kakraliya *et al.*, 2021). Consequently, indirect selection for plant height (PH), kernel rows per ear (NR), and fresh/field weight (FW) can be effective strategies to enhance grain weight (GW) in maize breeding programs (Aruna *et al.*, 2020; Purna *et al.*, 2024).

The heat map indicates that yield-related traits such as GW, FW, NK, EL, and ED were generally positively associated, which suggests that improvement in ear and kernel traits may contribute directly to higher yield. The strong linkage among flowering and maturity traits also reflects coordinated developmental timing. Negative or weak correlations were mostly non-significant, indicating limited trade-offs among most traits in this set.

Conclusion

The study demonstrated that flowering, maturity, plant-architecture, and yield-related traits in maize under charcoal rot stress are strongly and predominantly positively interrelated. Days to anthesis and days to silking were very highly correlated and closely associated with days to maturity, indicating coordinated phenology that supports efficient pollination and higher grain yield. Plant height exhibited strong positive relationships with ear height, ear length, and ear diameter, as well as with kernel rows per ear, kernels per row, and yield components, underlining its usefulness as an indirect selection trait for yield improvement. Ear length and ear diameter were strongly linked to kernel number per row and grain weight, confirming their importance as major yield-determining characters. The close association between field weight and grain weight suggests that field weight can reliably represent yield in breeding evaluations. While phenotypic correlations were higher than genotypic ones, indicating environmental influence, the significant genetic associations among plant height, kernel rows per ear, kernel number per row, and field weight provide a solid foundation for indirect selection. Incorporating these traits into breeding schemes can therefore enhance grain yield and charcoal-rot resistance in maize grown under stress-prone conditions.

Authors contribution: DSO: experimentation, data recording & drafting; **Rahul Kumar:** conceptualization, drafting editing; **SK:** conceptualization, drafting editing; **SD:** Data handling and drafting; **CGK:** experimentation, data recording, editing **SIH:** Experimentation, data recording and drafting; **AC:** Data handling and drafting; **PKB:** Experimentation, data recording and drafting; **RK:** Conceptualization, drafting & editing

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EFFECT OF WEED MANAGEMENT IN RICE CROP IN NORTHERN ZONE AREAS, YIELD AND QUALITY - A REVIEW

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Abstract

Rice (*Oryza sativa* L.) is the most important staple food crop for more than half of the world's population, including regions of high population density and rapid growth. In India, rice is the most extensively grown food crop, being cultivated on an area of about 43.42 million hectare with total production of 119.93 million tonnes and an average productivity of 2390 kg ha⁻¹. India is first in area under rice, while China is first in production. It provides about 21 per cent of the total calorie intake of the world population. Yield losses due to weeds vary with system of rice culture, variety, plant population, fertilizer applied, duration and time of application; weed species, amount of weed growth, season, ecology and climatic conditions. Weed management is an important key factor in obtaining higher crop yield. Reduction in yield to the tune of 34% in transplanted rice, 45% in direct seeded low land rice and 67% in upland rice are reported. Weeds compete for an adequate supply of the nutrients, moisture, light and the growing space. Successful weed management for rice more important to exploit its maximum production potential. Different weed management practices were studied in rice in present study were pretilachlor + bensulfuron methyl 70 g + 500 g a.i ha⁻¹ as pre emergence followed by mechanical weeding at 30 DAS/T, bispyribac sodium @ 50 g a.i ha⁻¹, farmer's practice and weedy check. among weed management practices, pre emergence application of bensulfuron methyl + pretilachlor mixture followed by mechanical weeding at 30 DAS/T resulted in higher B:C ratio compared to other methods of weed control.

Keywords: Transplanted Rice, Weed density, grain yield quality and herbicide.

Introduction

Rice (*Oryza sativa* L.) serves as the staple food for more than half of the world's population. Among cereals, rice is the most important and extensively cultivated crop across tropical and subtropical regions. Globally, it is grown on approximately 154 million hectares, producing around 500 million tonnes annually, with an average productivity of 3.9 t ha⁻¹. In India, rice is

cultivated across 44 million hectares, yielding approximately 137.8 million tonnes annually, with an average productivity of 2.9 t ha⁻¹. Rice is grown year-round in various parts of India, spanning diverse ecological zones over 43.8 million hectares (Jagtap *et al.*, 2012), contributing 85.3 million tonnes to national production. However, the average productivity in India remains relatively low at about 1.71 t ha⁻¹. Several factors contribute

to this low productivity, with weed infestation being one of the most critical and widespread biological constraints affecting crop yields. Weeds significantly hinder cereal crop growth, especially in rice fields. Uncontrolled weed growth in upland and lowland rice systems can result in yield losses ranging from 12% to as high as 81% (Chopra and Chopra, 2003; Mukherjee and Singh, 2005). Direct-seeded rice experiences 50–60% losses, while transplanted lowland rice can lose 70–80% of its yield due to weed competition. Effective weed management is therefore essential and poses a major challenge for rice cultivation. This chapter explores various weed management strategies—including mulching, tillage, manual weeding, herbicide application, allelopathy, crop rotation, intercropping, and preventive measures. It emphasizes how the optimal and judicious integration of these practices not only boosts productivity but also promotes environmental sustainability, offering significant benefits to farmers and agricultural ecosystems. (Umair Riaz *et al.*, 2020) .

Weed flora in rice field

Rice field is colonized by terrestrial, semi aquatic or aquatic depending on the types of rice culture and season. In recent years, rice production has increased with the introduction of high yielding varieties, but their maximum yield potential has not been fully realized owing to improper weed management.

Grasses	Sedges	Broadleaves
<i>Echinochloa colona</i>	<i>Cyperus iria</i>	<i>Monochoria vaginalis</i>
<i>E. crusgalli</i>	<i>Cyperus difformis</i>	<i>Ludwigia parviflora</i>
<i>Cynodon dactylon</i>	<i>Cyperus esculentus</i>	<i>Ammannia baccifera</i>
<i>Paspalum distichum</i>	<i>Scirpus supinus</i>	
<i>Panicum dichotomiflorum</i>	<i>Fimbristylis miliacea</i>	

Reported by (Singh *et al.*, 2007), (Ravisankar *et al.*, 2008) and Reddy (2010).

Weed competition

Weeds are recognized as major biological constraints that hinder the attainment of optimal rice productivity (Kumar and Ladha 2011, Rao and Nagamani 2013) and quality. It is estimated that every year, weeds cause yield losses from 15 to 76% in rice crop (Singh *et al.*, 2004, Mondal *et al.*, 2005, Rao and Nagamani 2010, Mishra *et al.*, 2012, Mandal *et al.*, 2013). Direct yield loss has

been estimated to the range from 16-86% depending on type of rice culture, cultivars, weed species and density, cropping season, plant spacing, fertilizer rate, duration and time of weed infestation and climatic and environmental conditions (Duany *et al.*, 2004, Kolay 2007). It is well established that weeds remove considerable quantities of nutrient from rice crop field. Estimate showed that weeds can deprive the crops by 47% N, 42% P, 50% K, 39% Ca and 24% Mg of their nutrient uptake thereby reduce the yield potential of rice (Balasubramanian and Palaniappan 2001). Hence, timely and effective weed control was essential for obtaining higher yield of rice (Sathyamoorthy *et al.* 2004, Kumar *et al.*, 2007). Nutrient removal by weeds has been reported to be about 21-42 kg N, 10-13.5 kg P and 17-27 kg K/ha in transplanted rice depending upon the soil, condition of cropping and location of growing rice (Sudhalakshmi *et al.* 2005, Puniya *et al.*, 2007b, Gowda *et al.*, 2009). In rain fed lowland rice, a period of 30-60 days after sowing was considered as critical period for crop weed competition to avoid grain yield losses (Moorthy and Saha 2005). The critical period of crop-weed competition in lowland transplanted and direct-seeded rice were 30-60 DAT and 40-60 DAS respectively (Das *et al.*, 2012).

Biology and ecology of major weeds

Ecological and biological studies of major weed species in transplanting rice system help identifying the susceptible stage in life cycle to make better strategy for effective weed management. *Ludwigia parviflora* is one of the most dominant species in different rice ecosystems throughout rice growing areas in Eastern and western India. The probable reason for its wide ecological amplitude might be the adaptation by special structure like periderm and pneumatophores as reported by Mukhopadhyay and Duany (1999) and Duany and Mukherjee (2013). Recently, the increased use of tillage implements (power tiller and tractor-drawn) has aggravated the problem of weeds by cutting into pieces which further grow and multiply as separate individuals (Duany and Mukhopadhyay 2004, Duany 2014b). In nursery bed, *Echinochloa crusgalli* emerges simultaneously with rice and owing to mimicry, it is transplanted simultaneously with rice seedlings in main field.

Losses caused by weeds

Crop yield losses due to weeds mainly depend upon their intensity as well as type of weed flora. There is a linear correlation between yield loss and population of weeds, however, above certain population limits; yield reduction becomes nearly constant due to self competition among weed plants. Grassy weeds were heavy competitors with rice crop and were followed by sedges and broad leaved weeds (Umapathy and Sivakumar, 2000). The greatest loss caused by the weeds resulted from their competition with crop for growth in terms of nutrients, soil moisture, light, space, etc.. (Walia, 2006). Chauhan and Johnson (2010) stated that when direct seeded rice was grown together with either jungle rice or *Ludwigiasps.* shoot competition reduced the growth and yield of rice more than root competition and rice grain yield was highly correlated with above and below ground biomass of the weeds.

Weed control methods

Weed control methods can be broadly categorized into cultural, mechanical, chemical, and biological approaches, each with its own set of techniques. Integrated Weed Management (IWM) combines these methods for more effective and sustainable control.

Cultural methods

Transplanting is the most old and popular method of rice establishment which is effective in reducing weed density. Puddling and submerged conditions under transplanting reduce weedgermination. The risks of crop yield loss due to competition from weeds in direct-seeded rice is greater than in transplanted rice because the weeds emerge concurrently with rice and farmers are not usually able to use standing water to suppress weeds at the early growth stages (Chauhan and Johnson 2010a). Transplanting of rice experiences lowest weed competition thereby records the lower weed population and dry weight (Prasad *et al.*, 2001, Singh *et al.*, 2007a, Mishra *et al.*, 2009) as compared to sowing of sprouted seeds in puddled condition and dry drilling of seeds and SRI. Intensive puddling with continuous submergence was very effective in reducing the weed dry weight (Subramanyam *et al.*, 2007). Azolla intercropping (dual cropping) significantly

reduced the weed density (Singh 2000, Biswas *et al.*, 2005). Flooding is one of the most important weed management options in lowland rice as many weeds will not germinate in anaerobic conditions. It is the timing, duration and depth of flooding that determines the extent of weed suppression by flooding (Mortimer *et al.*, 2005). But its effect on weeds is species specific (Chauhan and Johnson 2010b, Singh 2010). However, it is hardly possible to maintain water depth in rainy season. Two cultural practices which are often not given adequate attention in most of the farmer's field are through land preparation and proper land levelling. Land levelling should be an integral part of tillage operations because it is extremely important for good drainage in lowland rice ecosystems. An uneven field results in poor rice emergence in low spots and enhanced weed growth in high spots. In many areas, farmers do not take much care in levelling the land. Weed competitive rice cultivars are one of the integral parts of integrated weed management in transplanted rice.

Manual weeding

Interculture with khurpa, kasso/a, wheel hoe or tractor-drawn implements can be used for controlling weeds from different crops. This method is, no doubt very safe, but it is highly risky, less effective and efficient as compared to chemical methods. Sometimes, due to continuous rains or non-availability of farm labour, this method cannot be adopted at proper time. Morphologically similarity of weeds and crops makes hand hoeing further difficult and less efficient. However, mechanical practices can be adopted by unskilled labour and are invariably economical, non polluting, without residue problems, and relatively safe for operator. In rice field proper puddling at initials stage help to deteriorate population of major weed drastically and also help in moisture conservation (Mukherjee, 2004 b).

Mechanical weeding

Mechanical weeding is accomplished through incorporation of weeds in situ may help in effective recycling of the depleted nutrients. Rotary weeder was effective in controlling the weeds present in inter row space, but failed to control the weeds in intra row space or those in the vicinity of the crop.

Randriamiharisoa (2002) noticed that the mechanical weeding using rotating hoe with small toothed wheels increased the soil pores so that roots and microbes could more easily gain access to oxygen and also significantly increase the tiller production. Thiyagarajan *et al.*, (2002) reported that the use of Cono weeder resulted in 10 per cent grain yield increase during wet season while the yield increase was only three per cent higher in dry season than conventional method of weeding. Sudhalakshmi *et al.*, (2005) pointed out problems are encountered in incorporation of weeds like *Cynodon* and sedges with underground stolons and rhizomes which result in faster regeneration under mechanical weeding. Cono weeding alone was found to contribute 17.43 per cent for grain yield when the average grain yield under the cono weeding treatments 3376 kg ha⁻¹ was compared against the average grain yield under hand weeding treatments 2875 kg ha⁻¹ (Sreedevi, 2006). Mrunalini and Ganesh (2008) opined that the implements like cono weeder that helped to save labour, time and reduced man days required for weeding from 30 to 10 as they become more experienced in handling the cono weeder implement.

Chemical weed control

Herbicide usage is one of the most labour saving innovations (Moody 1993). Herbicide application has increased significantly over the last decade due to labor shortages, low herbicide prices, increased herbicide effectiveness and other advantages. A number of herbicides has been tested since last two and half decades for the management of weeds in transplanted rice with different times of application like pre-emergence, early post-emergence or post emergence in India. Wide range of herbicides are available for the management of grassy weeds (pretilachlor, butachlor, anilofos, fenoxaprop and oxadiargyl) as well as broad-leaved weeds (metsulfuron, chlorimuron, ethoxysulfuron and 2, 4 D). Pre-emergence herbicides in rice have very narrow application window and need continuous stagnation of water for their efficacy. Many times due to various constraints at farm level, the application of herbicides in the early growth stages is not possible and continuous use of same herbicide might cause resistance in weeds. Under

such situation, the post emergence herbicides are another option (Puniya *et al.*, 2007a). Sulfonylurea group is one of the most important classes of herbicide that has become popular all over the world which represent high level of activity, application flexibility, excellent selectivity and low mammalian toxicity even at very low dose with broad spectrum of weed control (Mukherjee and Singh 2005, Saha 2006, Saha and Rao, 2009, Duary, 2014a). Application of metsulfuron-methyl (10%) + chlorimuron-ethyl (10%) as early post-emergence showed better weed control efficiency against broad leaved and sedges as compared to grasses (Singh *et al.*, 2007b, Saha and Rao 2009, Pal *et al.*, 2009b). Chopra and Chopra (2003) found that application of pyrazosulfuron ethyl @ 20 or 25 g ha⁻¹ at 3 or 10 DAT significantly reduced the weed density and weed dry matter in transplanted rice during kharif season. Similar results were obtained by Angiras and Kumar, (2005). Shekar *et al.*, (2004) observed that significant increase in the grain yield (4.45 t ha⁻¹) by the application of pyrazosulfuron ethyl @ 20 g ha⁻¹ at 10 DAT. Saha (2006) reported that sulfonylurea group of herbicides is one of the most important classes of herbicide that has become popular all over the world which represent high level of activity, application flexibility; excellent selectivity and low mammalian toxicity even at very low dose with broad spectrum of weed control. Many times due to various constraints at farm level, the application of herbicides in the early growth stages is not possible and continuous use of same herbicide might cause resistance in weeds. Under such situation, the post emergence herbicides may another option (Puniya *et al.*, 2007b).

Herbicide mixtures

From research findings in the recent years, it was observed that combination of different herbicides with other standard herbicides even at lower dose proved more effective against a broad spectrum of weeds in transplanted rice. It has been observed that individual herbicides like metsulfuron methyl, chlorimuron ethyl, butachlor, anilofos, 2, 4-DEE, which are effective on certain weed species fail to control other weed species. Hence, to have a broad spectrum of weed control in single application, herbicide mixtures (both

concocted and tank) were tried and found to be effective. The simultaneous application of more than one herbicides in a mixture is increasingly becoming a standard practice in modern weed control strategies, particularly in absence of effective broad-spectrum herbicides to control highly diverse weed populations. A grassy herbicide in combination with broad-leaved weeds killer would take care of both types or grass killer in combination with herbicide that control both sedges and broad-leaved weeds will give a wider spectrum of weed control. Moody (1982) while reviewing the weed management rice, opined that herbicide combination usually provided wider control spectrum and/or better or more lasting control than when components of the combination are applied alone. Ethoxysulfuron + anilofos (0.02+0.375) kg ha⁻¹ as postemergence application and hand weeding twice were equally effective in recording the number of panicles per m² and grains per panicle (Bhowmick *et al.*, 2000). Maximum rice grain yield was recorded with a mix + 2,4-DEE (15 + 500g ha⁻¹) treatment and it was on par with hand weeding (Mukherjee and Singh, 2005). Puniya *et al.*, (2008) reported that the highest nitrogen and phosphorus uptake by the rice crop was recorded with application of triasulfuron + pretilachlor (0.009 + 0.5 kg ha⁻¹). Sequential application of butachlor two days after sowing and metsulfuron methyl + chlorimuron-ethyl 21 DAS recorded significantly lower density of weeds (Gopinath and Kundu, 2008).

Integrated weed management

Management system of weed populations aiming to keep infestation levels below those causing economic injury by combining any two or more of preventive, cultural, chemical biological methods. The definition implies that IWM is largely a decision or making process involving 1) when to apply control measures with use of critical thresholds and 2) what combination will provide best control at greatest profits. Integrated weed management is a system approach. Different methods viz cultural, biological, physical, chemical and ecological are employed to keep the population

of harmful organisms below the threshold level of economic damage. Control of weeds is achieved through a long-term management approach; using several weed management techniques. Mishra and Singh (2007) opined that pre emergence application of Pretilachlor @ 750g ha⁻¹ followed by one hand weeding at 30 DAS significantly reduced the weed population compared to weedy check during kharif season. Ravisankar *et al.*, (2008) noticed that the pre emergence application of pretilachlor plus @ 0.30 kg ha⁻¹ at 2DAS + hand weeding at 45 DAS registered lower weed density and higher weed control efficiency. Lakshmi *et al.*, (2009) concluded that supplementation of hand weeding at 20 DAS to pre emergence herbicides were found to be superior in reducing weed density and weed dry matter. Integrated use of herbicide azimsulfuron or pyrazosulfuron-ethyl with hand weeding at 40 DAT or butachlor and anilofos along with closer planting was effective for reducing weed population and dry weight (Gogoi *et al.*, 2001, Mondal *et al.*, 2005). Effective weed control in terms of reduced weed density and dry weight was achieved by pretilachlor with safener combined with Sesbania intercropping and Azolla dual cropping in wet-seeded rice (Subramanian and Martin 2006).

Conclusion

It is found from the above review, it can be concluded that Weeds significantly impact rice crops, competing effectively for resources up to 35-45 Days after Transplanting (DAT). This competition can lead to substantial grain yield reductions, ranging from 15% to 76%. Chemical weed control is getting very importance in rice field areas, where labour is less and costly. Some of the herbicides either alone or their combinations at lower dose have been proved economically viable alternative to hand weeding in management of weeds in rice field. However presently new concept integrated weed management is coming into existence. In this use of all suitable management technique are utilized in such a compatible way as to reduce weed population below economic threshold levels without deteriorating environment quality.

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FIELD EFFICACY OF DIFFERENT BIOPESTICIDE FORMULATIONS AGAINST DROSICHA MANGIFERA (GREEN)

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Abstract

Mango mealybug, *Drosicha mangiferae* (Green) is a serious, dilapidating, polyphagous, dimorphic and notorious pest of mango crop in Indian sub-continent. In an attempt to manage this pest different formulations of biopesticides viz., Azadirachtin (T₁), Aavya (T₂), Neem soap (T₃), Pongomia Soap (T₄), three concentration of *Metarhizium anisopiliae* (T₅, T₆ and T₇) and Imidacloprid 17.8SL as standard check (T₈) were tested under field conditions during the year 2021 and 2022. The post hoc analysis of data pertaining to field efficacy of biopesticide treatments suggested that Azadirachtin (2.64±0.59 and 2.85±0.64/panicle/twig) and Neem soap (3.37±0.71 and 3.63±0.75/panicle/twig) was found most effective against mealy bug population. Among three different concentration of microbial biopesticide, *M. anisopiliae* at a concentration of 0.1% recorded lowest mean insect population (4.06±0.42 and 4.38±0.45/panicle/twig) during both experimental years.

Key Words: Mango; *Drosicha mangiferae* (Green); Biopesticides

Introduction

Horticulture is a vital and rapidly growing sub-sector of agriculture, contributing about 28 percent to the Indian economy. Fruits and vegetable crops account for nearly 90% of the country's total horticultural production. Ramaiah, V. C. (2022). Among fruits, India is the leader in mango production (20772 thousand MT) which accounts for more than 40 per cent of global mango production (MoA&FW, 2022). It is grown across diverse agro-climatic conditions, and mealy bugs (*Drosicha mangiferae* Green) (Hemiptera:

Margarodidae), pose serious challenges to mango cultivation in the Indian sub-continent (Akhter *et al.*, 2022). It adversely influences fruit yield both quantitatively and qualitatively due to its infestation by nymphs and female bugs on roots, tender leaves, twigs, inflorescence and fruits. Additionally, their excretion of honeydew leads to the subsequent development of sooty mould. (Tandon and Lal 1978). Regular and indiscriminate use of insecticides to manage insect pests has resulted into adverse effect on human beings and their environment. (Patel and Kumar,

2020). Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. In India, the consumption of biopesticides produced from plant-derived is less than 1% and is only 12% globally. For sustainable farming, National Farmer Policy 2007 has promoted the utilization of biopesticides (Dar *et al.*, 2014). The most beneficial advantages of biopesticides are that they are eco-friendly and biodegradable. The awareness and demand for pesticide free products is increasingly expanding throughout the world. Thus, sustainability in agriculture with minimal use of harmful pesticides has been encouraged.

Materials and Method

The present investigation on efficacy of various biopesticide against *Drosicha mangiferae* (Green)

was laid out in a Randomized Block Design (RBD) with nine treatments and three replications at the Horticulture Research Centre, G.B. Pant University of Agriculture and Technology (29.50°N, 79.30°E). The recommended orchard management practices were followed and Fifteen years old mango trees (cv. Dasher) were randomly selected, all of which had uniform flowers and shapes. The first spray of various biopesticides was applied when mealy bug nymphs infesting mango inflorescence in the month of March, followed by the second and third application at weekly interval. All treatments were applied using a foot-operated sprayer. Mealy bug population was recorded from 10 inflorescences one day before spraying (pre-treatment count) and at three and seven days after each application (post-treatment counts).

Details of Biopesticides used in the field experiment

Treatment No.	Biopesticide Details	Group Name	Formulation	Dosage	Source
T ₁	Azadirachtin	Botanical	EC (3000ppm)	3ml/l	Local Market
T ₂	Aavya	Botanical	Soap	4ml/l	IIHR, Bangalore
T ₃	Neem Soap	Botanical	Soap	10g/l	IIHR, Bangalore
T ₄	Pongomia soap	Botanical	Soap	10g/l	IIHR, Bangalore
T ₅	M. anisopliae	Microbial	Oil- based (1×10 ⁸ cfu/ml)	0.25ml/l	IIHR, Bangalore
T ₆	M. anisopliae	Microbial	Oil- based (1 × 10 ⁸ cfu/ml)	0.5ml/l	IIHR, Bangalore
T ₇	M. anisopliae	Microbial	Oil- based (1×10 ⁸ cfu/ml)	1ml/l	IIHR, Bangalore
T ₈	Imidacloprid (Standard check)	Neonicotinoid	17.8 SL	0.30ml/l	Local Market
T ₉	Control	—	—	—	—

Statistical Analysis

The efficacy data for various biopesticides against *Drosicha mangiferae* (Green) were analyzed using a Randomized Block Design (RBD). The collected data were subjected to analysis of variance (ANOVA) using the statistical software SPSS (version 23.0). Significant differences among the means of different treatments were tested using Tukey's test.

Percent reduction of insect population

Percent reduction of *Drosicha mangiferae* t in mango was computed using Henderson-Tilton's (1955) formula:

Corrected %

$$1 \frac{n \text{ in Control before treatment} - n \text{ in T after treatment}}{n \text{ in Control after treatment} - n \text{ in T before treatment}} \times 100$$

Where: n= Insect population, T= Treated

Results and Discussion

A total of four botanicals viz., Azadirachtin, Aavya, Neem soap, Pongomia soap and three concentration of oil based formulation of *Metarhizium anisopliae* (0.25, 0.50 and 1ml per litre of water) were evaluated against *Drosicha mangiferae* (Green) under the field conditions. All the treatments significantly reduced the mealy bug population in both the experimental years. The highest number of mealy bug was found on the untreated trees in both the season. Although the insect populations were slightly lower during 2021 than 2022. The use of Imidacloprid 17.8SL (Confidor) resulted in the best overall performance with lowest overall mean population and 72.99 to 75.10 (Fig:1) per cent reduction in mealy bug population over control.

The results on the effect of different biopesticide treatments against mango mealy bug are presented in Table 1, Table 2, and Fig. 1. Overall mean results suggested that, Azadirachtin was found most effective treatment among biopesticides with a mean population of 2.64 ± 0.59 and 2.85 ± 0.64 mealy bug per panicle during 2021 and 2022. These results were statistically similar with Neem Soap (T_3) having 3.37 ± 0.71 and 3.63 ± 0.75 mealy bug per panicle during both the years, respectively. Moreover, the treatments *viz.*, Aavya, *M. anisopila* (1ml per litre of water), Pongomia soap and *M. anisopila* (0.5 ml per litre of water) were statistically at par with each other with a mean population ranging from 3.88 ± 0.58 to 4.93 ± 0.54 in 2021 and 4.40 ± 0.63 to 5.31 ± 0.60 mealy bug per panicle in 2022. It was observed that *M. anisopila* (0.25 ml per litre of water) was least effective and exhibited highest mean pest count among all the biopesticide treatments over the entire period of study.

The overall performance of biopesticide treatments in order to their potential against mealy bug revealed that, Azadirachtin exhibited the highest efficacy with 72.61 and 70.73 per cent reduction over control during 2021 and 2022. Neem soap recorded 63.52 to 65.08 percent

reduction in pest population. Subsequently, Aavya was found third most effective treatments and attained 59.80 and 55.74 per cent reduction over control. Among the three concentration of microbial pesticide, *M. anisopila* (1ml/litre) formulation showed 57.94 and 56.76 per cent decrease in mealy bug population during both the years, respectively. Pongomia soap and *M. anisopila* (0.50 and 0.25 ml/litre) apparently showed poor results against mealy bug infestation.

The results were in agreement with Lanjar *et al.* (2015) who reported superiority of azadirachtin and neem leaf in reducing mealy bug population as compared to other plant extract *viz.*, Eucalyptus, Dhatura and Akk. These results were in close agreement with Nebie *et al.* (2018) who reported that, *M. anisopila* showed higher mortality rate at higher doses after three application while insecticide was found more potent than biopesticides against mealy bug infestation. Manjushree and Chellappan (2019) concluded that highest decrease in mealy bug population was observed in quinalphos (96.73%) followed by azadirachtin (87.75%). Moreover, *M. anisopila* showed 59.29 and 72.72 per cent reduction in mealy bug population after 1st and 2nd application, respectively.

Table 1: Efficacy of various biopesticide against *Drosicha mangiferae* during the year 2021

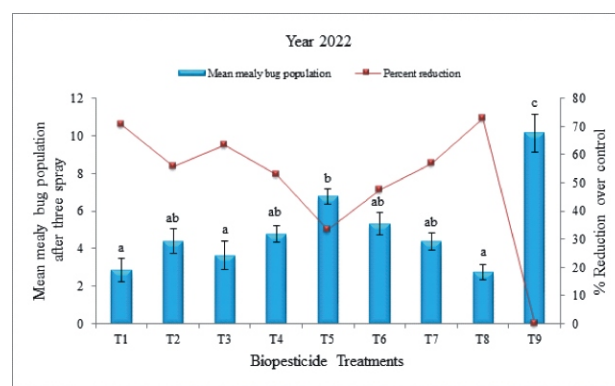
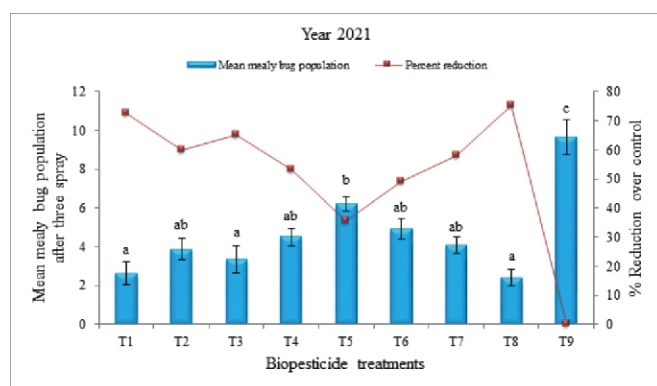
Treatments	Biopesticides	Dose (ml/g per lit. water)	Average number of mealy bug nymph/adult population per panicle/ twig							Overall Mean	Per cent reduction over control
			Pre count	Days after first spray		Days after second spray		Days after third spray			
					3DAS	7DAS	3DAS	7DAS	3DAS		
T ₁	Azadirachtin	4ml	9.95±0.46	3.61±0.87	4.78±1.16	2.04±0.41	2.86±0.57	1.21±0.24	1.35±0.27 1	2.64±0. 59a	72.61
T ₂	Aavya	4ml	8.47±0.97	4.42±0.81	5.84±1.08	3.73±0.64	4.28±0.37	2.40±0.28	2.59±0.3 0	3.88±0. 57ab	59.80
T ₃	Neem soap	10g	9.70±1.00	3.98±0.82	5.26±1.10	2.60±0.49	2.31±0.59	2.96±0.60	3.10±0.6 3	3.37±0. 71a	65.08
T ₄	Pongomia soap	10g	8.25±0.65	5.10±0.50	6.46±0.63	4.89±0.45	3.65±0.31	3.33±0.30	3.62±0.3 3	4.51±0. 43ab	53.26
T ₅	M. anisopilae	0.25ml	9.17±0.79	8.40±0.38	7.74±0.35	6.55±0.30	5.52±0.41	4.87±0.36	4.26±0.3 1	6.22±0. 36b	35.48
T ₆	M. anisopilae	0.5ml	8.61±1.09	8.33±0.91	7.16±0.78	4.77±0.52	3.85±0.42	2.89±0.31	2.55±0.2 7	4.93±0. 54ab	48.94
T ₇	M. anisopilae	1ml	9.08±1.20	8.64±0.90	6.89±0.71	3.91±0.40	2.27±0.23	1.36±0.1 3	1.27±0.1 4	4.06±0. 42ab	57.94
T ₈	Imidacloprid 17.8 SL*	0.30ml	9.50±1.37	2.18±0.56	1.36±0.34	2.54±0.37	5.83±0.80	1.73±0.2 4	0.78±0.1 1	2.40±0. 40a	75.10
T ₉	Control	–	8.70±0.17	8.95±0.89	8.39±0.97	9.50±0.98	11.14±1.0 3	9.77±0.8 4	11.29±0. 52	9.65±0. 88c	-
	C.D. (5%)		N/S	0.608	0.927	0.624	0.763	0.663	0.499		
	SE(m) ±		0.378	0.201	0.307	0.206	0.252	0.19	0.165		
	C.V. (%)		7.205	5.978	8.870	7.935	9.435	11.200	8.348		

*Imidacloprid 17.8 SL as a standard check

Table 2: Efficacy of various biopesticide on the incidence of mango *Drosicha mangiferae* during the year 2022

Treatments	Biopesticides	Dose (ml/g per lit. water)	Average number of mealy bug nymph/adult population per panicle/ twig								Overall Mean	Per cent reductio n over control
			Pre count	Days after first spray		Days after second spray		Days after third spray				
				3DAS	7DAS	3DAS	7DAS	3DAS	7DAS			
T ₁	Azadirachtin	4ml	10.53±1.39	3.90±0.94	5.52±1.33	2.46±0.41	3.37±0.64	1.51±0.30	1.02±0.20	2.85±0.64a	70.73	
T ₂	Aavya	4ml	9.40±0.43	4.77±0.87	6.13±1.13	4.53±0.43	4.85±0.54	2.86±0.32	3.73±0.43	4.40±0.63ab	55.74	
T ₃	Neem soap	10g	10.34±0.59	4.30±0.90	5.92±1.05	2.88±0.69	2.56±0.51	3.70±0.74	2.79±0.56	3.63±0.75a	63.52	
T ₄	Pongomia soap	10g	10.13±1.28	4.96±0.48	6.78±0.66	5.42±0.37	4.05±0.37	4.16±0.38	3.26±0.30	4.77±0.43ab	52.86	
T ₅	M. anisopilae	0.25ml	10.81±0.49	9.07±0.41	8.12±0.37	7.26±0.58	6.13±0.44	6.09±0.46	3.83±0.27	6.75±0.41b	33.32	
T ₆	M. anisopilae	0.5ml	9.79±0.23	8.96±0.98	7.41±0.82	5.29±0.67	4.27±0.47	3.61±0.39	2.30±0.25	5.31±0.60ab	47.59	
6T ₇	M. anisopilae	1ml	10.02±0.39	9.33±0.97	7.23±0.75	4.33±0.45	2.52±0.26	1.70±0.17	1.14±0.11	4.38±0.45ab	56.76	
T ₈	Imidacloprid 17.8 SL*	0.30ml	10.66±1.33	1.85±0.47	2.40±0.34	3.81±0.52	5.47±0.75	2.16±0.30	0.70±0.09	2.73±0.42a	72.99	
T ₉	Control		9.25±0.92	9.80±0.97	8.54±0.90	9.23±1.06	10.36±1.19	11.21±0.97	13.32±0.92	10.12±1.00c	-	
	C.D. (5%)		N/S	0.735	1.003	0.649	0.822	0.754	0.783			
	SE(m) ±		0.493	0.243	0.331	0.214	0.272	0.249	0.259			
	C.V. (%)		8.466	6.826	8.971	7.546	9.784	10.504	12.586			

*Imidacloprid 17.8 SL as a standard check



T₁ Azadirachtin, T₂ Aavya, T₃ Neem soap, T₄ Pongomia soap, T₅ M. anisopilaie (0.25ml), T₆ M. anisopilaie (0.50 ml), T₇ M. anisopilaie (1ml), T₈ Imidacloprid 17.8% SL, T₉ (Water),

Fig 1: Field effectiveness of biopesticide treatments against *Drosicha mangiferae* during 2021 and 2022

Conclusion

The results of this study highlight the crucial role of the novel biopesticide formulations, particularly Azadirachtin, Neem soap, Aavya, and *M. anisopliae* (1 ml/L) achieved 56.76 to 72.61 per cent reduction in mealy bug populations when applied three times at weekly intervals. These biopesticides can be effectively used as preventive measures to manage mealy bug infestations when pest levels are low.

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EFFICACY OF PACKAGING FILMS AND CHEMICAL PRESERVATIVES IN EXTENDING POST-HARVEST VASE LIFE AND BIOCHEMICAL QUALITY OF CUT CHRYSANTHEMUM (*CHRYSANTHEMUM MORIFOLIUM* RAMAT) FLOWERS

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Abstract

Cut chrysanthemum (*Chrysanthemum morifolium* Ramat) is one of the world's two most valuable flower crops. It has substantial cut flower decline and death rates (25%-40%) due to fast flower decline, occlusion of the xylem, and lack of water for the cut flower to uptake via the xylem. Careful application of packaging materials and floral preservatives as a whole may provide smallholders and producers with cost-effective ways to extend the postharvest period of flowers. There is limited data available characterizing the benefits of film types versus the advantages of floral preservative chemicals, under subtropical Indian storage conditions. Thirteen different treatment combinations were evaluated using a completely randomised design with three replications and involved;

- i. five different types of film (polypropylene, low density polyethylene, corrugated cardboard, metallised BOPP (biaxially oriented polypropylene), and an open/control),
- ii. five different types of preservatives (sucrose, sucrose + 8-HQC (8-hydroxyquinoline citrate), aluminium sulfate, silver nitrate, salicylic (acid)), and
- iii. combined film and preservative treatments. Assessment included evaluation of: Cut stems of cv. 'Snowball' were assessed at 20 ± 2 °C for vase life, fresh weight retention, stem water uptake, relative water content, chlorophyll content (SPAD), flower diameter retention, stem length retention, malondialdehyde (MDA), total soluble protein, antioxidant enzyme activities (SOD, POD, CAT), vase solution bacterial load, and overall quality score. The longest vase life of chrysanthemum cut flowers was found in Treatment T12 (LDPE Packaging + 3% Sucrose + 200 mg L⁻¹ of 8-HQC) with a mean of 16.8 ± 0.3 days representing a 127% increase over the control (7.4 days). In addition, Treatment T12 exhibited the highest fresh weight retention (94.6%), highest chlorophyll content (44.8 SPAD), highest amounts of antioxidant enzyme activity, lowest amounts of malondialdehyde (2.18 nmol g⁻¹ FW), and lowest microbial count (840×10^6 CFU mL⁻¹). Treatment T13 (mBOPP Packaging + Sucrose + 8-HQC) was rated as the next most effective combination (15.4 days). Among packaging-only treatments, LDPE (T3, 10.8 days) outperformed all other treatments. Among preservatives, the combination of Sucrose + 8-HQC (T7, 13.2 days) was superior to Silver Nitrate (T9, 12.6 days) with all comparisons being statistically significant ($P = 0.05$).

Therefore, packaging of chrysanthemum cut flowers with LDPE combined with a vase solution containing Sucrose and 8-HQC represents the optimal and commercially viable postharvest method for chrysanthemum cut flowers under conditions of subtropical storage due to the combined synergistic reduction of transpiration, bacteriologically caused xylem obstruction, oxidative senescence, and utilization of respiratory substrates.

Key word: Chrysanthemum, Post Harvest, antioxidant enzyme activities, malondialdehyde, total soluble protein, Preservatives

Introduction

Chrysanthemum flowers (*Chrysanthemum morifolium* Ramat) are one of the top five economically traded crops worldwide, with an estimated global wholesale value of more than 4.8 billion US dollars. Approximately 18 per cent of cut flower production in India is represented by chrysanthemum, with major growing areas located in Uttarakhand, Himachal Pradesh, Maharashtra and West Bengal. Approximately 120,000 smallholder farmers in India depend on this crop for their livelihoods, most of whom grow the flowers at elevations between 800 and 2,200 metres above sea level, where cool weather creates ideal conditions for producing high-quality flowers.

Postharvest losses in chrysanthemum are extremely high despite advances in agriculture/horticulture. Losses are estimated at 25-40% during the period of harvest to sale based on the supply chain in India (Reddy & Mishra 2021). The losses are due to a combination of physiological and biochemical mechanisms that have led to: (i) decreasing water levels in plants, due to vascular embolism and increased transpiration (ii) accelerated senescence through ethylene, whereby the expression of senescence-associated genes and chlorophyll is accelerated (iii) microbial growth on the cut stem surface, resulting in occlusion of xylem vessels (iv) depletion of carbohydrate reserves that are required for cellular respiration and osmotic regulation (v) peroxidative damage to membranes associated with the accumulation of reactive oxygen species (ROS) (Bayat *et al.* 2021 ; Gao *et al.* 2022).

The primary function of packaging materials in postharvest management is to help change the environment of harvested crops' stems; this is done mainly by modifying how crops' environment interacts with their surroundings. Polymeric films such as low-density polyethylene (LDPE) and polypropylene (PP) provide a barrier against water vapor, an advantage when maintaining higher humidity levels. LDPE and PP also have been recorded to provide passive modified atmosphere properties to prevent microbial respiration and manage respiration slower than average compared with other materials (El-Tanany *et al.*, 2020; Zhou *et al.*, 2022). A potential benefit to using metalized biaxially oriented polypropylene (mBOPP) as a packaging material is that it combines properties of moisture barriers and light barriers as well as reflectivity to heat, which makes it a likely candidate as an additional beneficial packaging material when subject to temperature fluctuations commonly experienced in Indian transit conditions. Similarly, corrugated box packaging has been historically used as a traditional means of mechanical protection during shipping; however, this traditional packaging does not provide any barrier against the movement of water vapor through the structure, and these types of packaged goods also will exhibit susceptibility to structural collapse due to the actions of high humidity (Kumar *et al.*, 2020).

Flower preservatives applied via base solutions can help stop flowers from rotting after harvest, which can happen biochemically. Sucrose (2-5% w/v) is an energy source for flower respiration and keeps petals swollen with water by holding water pressure in the petals. Sucrose also delays the breakdown of proteins in the petal tissues. The biocide, 8-hydroxyquinoline citrate (8-HQC) works at 100-300 mg/L as an antibacterial agent for most bacteria by chelating metal ions and making the vase solution acidic (pH 3.5-4.0) to prevent bacterial growth from creating biofilm formations that block water transport through the xylem. AgNO₃ and other silver compounds are both antibacterial agents and are also a competitive inhibitor to the ETR1 ethylene signalling receptor (Darras & Terry, 2021). The addition of aluminium sulphate will lower the pH of the vase solution to about 4.0-4.5 and produce an antibacterial effect while also improving water transport in the xylem

via vessel dilation due to low acidic pH (Sharma *et al.*, 2022). Similarly, salicylic acid (SA) regulates the antioxidant defence of flowers by increasing antioxidants like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) to decrease membrane damage from reactive oxygen species (Farouk & Al-Amri, 2020).

Comprehensive research on the synergistic effects of combining film packaging and chemical preservatives has been conducted only for chrysanthemum under Indian subtropical storage conditions due to the fact that previous research on these factors has focused on independent evaluation. The biochemical basis for the impact of treatment on chrysanthemum from combined film packaging and chemical preservatives has also received very little attention (Li *et al.*, 2021; Chen *et al.*, 2023), in particular the evaluation of antioxidant enzyme activity, lipid oxidation and proteins. Investigating how these mechanistic factors interact with one another is crucial to developing integrated cost-effective postharvest protocols designed specifically for the Indian smallholder producers.

Materials and Methods

Plant Material, Site, and Experimental Conditions

The researchers conducted their study from session 2023-24 and 2024-25 between January to March, at the Department of Agriculture, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut. The sample used for the experiment were flowers of couscous from the chrysanthemum cv. "Snowball", which is a large globular flower; this variety was chosen because it is widely grown and very susceptible to deterioration after cutting.

To prevent pre-harvest stress due to water

enrollments, we made sure that the stems that we were cutting were harvested when they were in their quarter open bud stage. We harvested these stems between the hours of 05:30 and 06:30 hours local time. We harvested the stems at a length of 50 ± 2 cm and at an angle of 45° using ethanol sterilised secateurs. We removed all leaves/foilage from the bottom 15 cm of the stems. The stems were re-cut in distilled water and placed into buckets with distilled water for a pre-conditioning period of 2 hours before treatments were imposed. All experimental units will be maintained at a temperature of 20 ± 2 °C and a relative humidity of 60 - 65 % in a natural diffuse light environment (photosynthetically active radiation: 12 to 18 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$) for the entire duration of assessment.

Experimental Design and Treatments

Thirteen treatments and three replications were assigned to each treatment using a Completely Randomised Design (CRD) during this study. Each experimental unit consisted of five cut stems placed into 500 mL glass vases containing 400 mL of the respective treatment solution; these vases were replenished to their original volume of 400 mL every day throughout the experiment. Table 1 contains detailed descriptions of the thirteen treatments used in this study.

Heat sealable packaging films (PP, LDPE, mBOPP: 25 micron), were heat sealed around each stem within 15 minutes post harvest, for the dry storage packaged treatment group. Fresh vase solutions were prepared daily, using distilled water as the diluent. 8-HQC (analytical grade from Sigma Aldrich, = 98%) was the other chemical used in addition to sucrose (food grade, 99.5%), aluminium sulphate, silver nitrate and salicylic acid. All glassware and secateurs were autoclaved following each observation period to reduce the possibility of cross-contamination.

Table 1. Experimental treatment schedule for postharvest evaluation of cut chrysanthemum cv. 'Snowball'. Thirteen treatments were evaluated in CRD with three replications.

Treatment Code	Treatment Description	Category	Active Ingredient	Dose / Concentration
T1	Distilled water (control)	Control	-	-
T2	Polypropylene (PP) film wrap + distilled water	Packaging	PP (25 μm)	-
T3	Low-density polyethylene (LDPE) wrap + distilled water	Packaging	LDPE (25 μm)	-
T4	Corrugated fibre board box + distilled water	Packaging	CFB (3-ply, 5 mm)	-

T5	Metalised BOPP wrap + distilled water	Packaging	mBOPP (20 m)	-
T6	Sucrose vase solution	Preservative	Sucrose	3% (w/v)
T7	Sucrose + 8-hydroxyquinoline citrate (8-HQC) vase solution	Preservative	Sucrose + 8-HQC	3% + 200 mg L ⁻¹
T8	Aluminium sulphate vase solution	Preservative	Al ₂ (SO ₄) ₃	200 mg L ⁻¹
T9	Silver nitrate vase solution	Preservative	AgNO ₃	50 mg L ⁻¹
T10	Salicylic acid vase solution	Preservative	Salicylic acid	150 mg L ⁻¹
T11	PP wrap + sucrose + 8-HQC combined	Combined	PP + Sucrose + 8-HQC	3% + 200 mg L ⁻¹
T12	LDPE wrap + sucrose + 8-HQC combined	Combined	LDPE + Sucrose + 8-HQC	3% + 200 mg L ⁻¹
T13	mBOPP wrap + sucrose + 8-HQC combined	Combined	mBOPP + Sucrose + 8-HQC	3% + 200 mg L ⁻¹

CFB = Corrugated Fibre Board; mBOPP = Metalised Biaxially Oriented Polypropylene; 8-HQC = 8-Hydroxyquinoline Citrate; SA = Salicylic acid. Concentrations are expressed on a volume-to-volume (w/v) or mass-to-volume basis as appropriate.

Postharvest Quality Observations

Data were collected at 48 hour intervals beginning on day zero (i.e., treatment imposed) and continued until the termination criteria was met. Measurements recorded included:

- **Vase life (days):** The number of days from treatment imposition until the point at which = 50% of petals were exhibiting wilting/browning/shattering, using the visual termination criteria defined by Nowak and Rudnicki (1990) and modified for chrysanthemums.
- **Fresh weight retention (%):** (Weight on day of measurement/initial weight) x 100. Fresh weight determined using a digital analytical scale (Mettler-Toledo ME204E, accuracy $\pm$ 0.0001 g).
- **Daily stem water uptake (mL per stem):** The volume of vase solution consumed per stem over a 24 hour period. Measured gravimetrically after correcting for evaporative losses from a blank vase of the same size covered in Parafilm.
- **The relative water content (RWC):** It was calculated using the following equation $RWC = [(FW - DW)/(TW - DW)] \times 100$; FW, DW and TW refer to petal discs of 1cm² area (FW = fresh weight, DW = dry weight [72 hours at 70°C] and TW = turgid weight [24 hours at 4°C rehydration]).
- **Chlorophyll content:** it was measured non-destructively on the 3rd fully expanded leaf from the apex using a SPAD-502Plus

Chlorophyll Meter (Konica Minolta, Japan), taking the mean of five readings per stem.

- **Retention of flower diameter (%) and stem length (%):** It were measured with a digital Vernier calliper (0.01 mm resolution) and standard graduated tape respectively and expressed as a percentage of initial values. Date of first visible browning of petals was recorded as the first date that brown discoloration appeared on any of the 12 petals of a capitulum.

Biochemical Analyses

Using a Shimadzu UV-1900 spectrophotometer, assays were conducted on fresh petal (0.5 g/sample) from randomly selected representative flowers at 0, 6 and 12 days and the corresponding flower's vase life. Assays were done on a UV/Vis Spectrophotometer at a specific wavelength in order to determine biochemical parameters from the fresh petal sample at each vase stage (Day 0 to 12).

Malondialdehyde content was measured by the TBARS assay developed by Heath and Parker (1968) using modified methods from Hasanuzzaman *et al.* (2023) and reported as nmol of MDA per gram of fresh weight (FW). The total soluble protein content was determined by the Bradford dye-binding procedure with the base of bovine serum albumin (BSA). The superoxide dismutase activity was measured based on NBT photoreduction inhibition; 1 unit of SOD was determined as the amount of enzyme required to provide 50% inhibition of NBT reduction. The peroxidase activity was measured using guaiacol

as the hydrogen donor, while the catalase activity was measured as the decomposition of H_2O_2 at 240 nm. All enzyme activities were reported as U/mg of protein (Li *et al.*, 2021).

Microbiological Analysis of Vase Solution

Counts were made of the bacterial populations in the vase solutions at day 0 (just after their placement), day 4, and day 8 using the serial dilution pour plate technique on nutrient agar (NA) incubated at 37 °C for 2 days. Colony forming units (CFU/mL) were counted and the counts converted to logarithmic values prior to performing statistical analyses. Taxonomic classification of the dominant genera of bacteria present is based on colony morphology, Gram-staining, and standard biochemistry tests as per Bergey's Manual of Systematic Bacteriology.

Overall Quality Score

The overall quality score (OQS) assigned to the chrysanthemums was a 1 to 5 scale based on the appearance of the flowers. On a 1 to 5 rating system; 1 = Completely dead, not sellable; 2 = Extreme wilting or browning (over 50% of petals affected); 3 = Some wilting/effectively borderline sellable; 4 = Lightly wilted/commercially acceptable; 5 = Full turgency/fresh/new-appearing; maximum sale value. Scores were evaluated independently by three trained scorers, and the mean score from these three individual scorers was used as the basis for analysis. The scoring design was based on a prior study of standard chrysanthemum quality by Bayat *et al.* (2021).

Statistical Analysis

Using OPSTAT (statistical software package from CCS Haryana Agricultural University), one way analysis of variance (ANOVA) was performed on the data collected from all four seasons to determine treatment means. Treatment means were separated at $p = 0.05$ using Duncan's Multiple Range Test (DMRT). The mean and standard error of the mean ($\text{SEm} \pm$) are presented for each season. After verifying the variance homogeneity via Bartlett's test ($p > 0.05$ for all parameters), the seasonal data were combined into one data set. A correlation analysis using

Pearson's correlation coefficients (r) was performed between the major postharvest characteristics in order to identify meaningful functional inter-relationships. Finally, Principal Component Analysis (PCA) was performed with XLSTAT 2024 to assess multiple variables among treatments.

Results

Vase Life, Fresh Weight Retention, Stem Water Uptake, and Relative Water Content

There were significant differences ($p = 0.05$) for vascular life and water relations between the thirteen treatments with every one having a value of $p = 0.05$ (Table 2). The treatment with the longest vascular life was treatment T12 (LDPE + sucrose + 8-HQC) which had a vascular life of 16.8 ± 0.3 days representing an increase of 127.0% over the control treatment T1 (7.4 ± 0.3 days). Treatment T13 (mBOPP + sucrose + 8-HQC) had the second longest vascular life at 15.4 ± 0.3 days with treatment T11 (PP + sucrose + 8-HQC) being third at 14.6 ± 0.3 days. Of the single preservative treatments, treatment T7 (sucrose + 8-HQC) had a vascular life of 13.2 days while treatment T9 (AgNO_3 , 12.6 days) and treatment T8 (Al_2SO_4 , 11.8 days) were not as long. The treatments that were packaging only that used LDPE (T3, 10.8 days) had a greater length of vascular life than PP (T2, 9.6 days), mBOPP (T5, 9.2 days), and CFB (T4, 8.9 days).

On the final evaluation day, the ranking of fresh weight retention remained the same as that of previous evaluations. T12 had the highest weight retention rate with $94.6 \pm 0.7\%$ of its original fresh weight and the control (T1) was the lowest with $68.2 \pm 1.4\%$ of its original fresh weight. For the analysis of variance, the combination of T12, T13, and T11 were statistically defined to be a homogenous group with superior characteristics (DMRT, $p = 0.05$). The stem water uptake results were consistent with other measurements, where T12 was measured to have $7.12 \pm 0.22 \text{ mL stem}^{-1} \text{ day}^{-1}$ and T1 with $3.12 \pm 0.18 \text{ mL stem}^{-1} \text{ day}^{-1}$. The amount of water taken up by T1 was consistently lower than T12 after day 4, illustrating increasing amounts of blockage in the xylem. Conversely, T12 maintained greater than

90% of water uptake capacity for the full 12 day period, indicating that the inclusion of 8-HQC maintained an adequate water status in chrysanthemum. RWC was similar to fresh weight retention, where T12 had an RWC of $95.2 \pm 0.8\%$ and T1 had an RWC of $72.4 \pm 1.2\%$. The days until first petal browning were maximised in T12=16.8 days, T13= 15.4 days, T11=14.6 days, and control=7.4 days. The correlation coefficient between vase life and fresh weight retention was determined to be 0.976 ($p < 0.01$), indicating that maintenance of tissue water content was the most significant factor that influenced the vase life of chrysanthemum.

Table 2. Effect of packaging materials and floral preservatives on vase life, fresh weight retention, stem water uptake, relative water content, and days to first petal browning of cut chrysanthemum cv. 'Snowball' (pooled over two seasons, 2023–24 and 2024–25).

Treatment	Vase Life (days)	Fresh Weight Retention (%)	Stem Water Uptake (mL stem ⁻¹ day ⁻¹)	Relative Water Content (%)	Days to First Petal Browning
T1 (Control)	$7.4 \pm 0.3g$	$68.2 \pm 1.4f$	$3.12 \pm 0.18f$	$72.4 \pm 1.2f$	$7.4 \pm 0.3g$
T2 (PP)	$9.6 \pm 0.4ef$	$76.5 \pm 1.3de$	$4.28 \pm 0.21de$	$78.6 \pm 1.4de$	$9.6 \pm 0.4ef$
T3 (LDPE)	$10.8 \pm 0.4d$	$80.3 \pm 1.2cd$	$4.86 \pm 0.22cd$	$82.1 \pm 1.3cd$	$10.8 \pm 0.4d$
T4 (CFB)	$8.9 \pm 0.5f$	$73.4 \pm 1.5e$	$3.74 \pm 0.19ef$	$75.8 \pm 1.5e$	$8.9 \pm 0.5f$
T5 (mBOPP)	$9.2 \pm 0.5ef$	$74.8 \pm 1.4e$	$3.96 \pm 0.20e$	$77.2 \pm 1.4e$	$9.2 \pm 0.5ef$
T6 (Sucrose)	$11.4 \pm 0.4d$	$82.6 \pm 1.1c$	$5.14 \pm 0.23c$	$84.3 \pm 1.2c$	$11.4 \pm 0.4d$
T7 (Suc+8-HQC)	$13.2 \pm 0.4c$	$86.8 \pm 1.0b$	$5.82 \pm 0.24b$	$88.1 \pm 1.1b$	$13.2 \pm 0.4c$
T8 (Al ₂ SO ₄)	$11.8 \pm 0.5d$	$83.4 \pm 1.2bc$	$5.28 \pm 0.22c$	$85.2 \pm 1.2bc$	$11.8 \pm 0.5d$
T9 (AgNO ₃)	$12.6 \pm 0.4c$	$85.2 \pm 1.1bc$	$5.64 \pm 0.23b$	$87.0 \pm 1.1bc$	$12.6 \pm 0.4c$
T10 (SA)	$11.2 \pm 0.5d$	$81.9 \pm 1.2c$	$5.04 \pm 0.22cd$	$83.6 \pm 1.2c$	$11.2 \pm 0.5d$
T11 (PP+Suc+8-HQC)	$14.6 \pm 0.3b$	$90.2 \pm 0.9a$	$6.38 \pm 0.24b$	$91.4 \pm 1.0a$	$14.6 \pm 0.3b$
T12 (LDPE+Suc+8-HQC)	$16.8 \pm 0.3a$	$94.6 \pm 0.7a$	$7.12 \pm 0.22a$	$95.2 \pm 0.8a$	$16.8 \pm 0.3a$
T13 (mBOPP+Suc+8-HQC)	$15.4 \pm 0.3ab$	$92.1 \pm 0.8a$	$6.74 \pm 0.23a$	$93.1 \pm 0.9a$	$15.4 \pm 0.3ab$
SEm $\pm$	0.18	0.54	0.11	0.48	0.18
CD ($p = 0.05$)	0.54	1.62	0.34	1.44	0.54

Values are mean $\pm$ SEm ($n = 3$ replications $\times$ 5 stems = 15 observations per treatment per season). Means within a column followed by different lowercase superscripts differ significantly at $p = 0.05$ (Duncan's Multiple Range Test). SEm $\pm$ = Standard Error of Mean; CD = Critical Difference at $p = 0.05$.

Chlorophyll Content, Flower Diameter Retention, Stem Length Retention, MDA Content, and Total Soluble Protein

There were major differences in the treatments for every measurement of biochemistry and morphology of the flower (Table 3). The greatest chlorophyll content (measured in SPAD units) was shown in treatment T12 (44.8 ± 1.2) followed by T13 (43.2 ± 1.1) and T11 (41.2 ± 1.1). The control treatment (T1) had the least amount of SPAD reading at the end of the vase life (27.4 ± 0.9) showing that the chlorophyll was used up at an accelerated rate. Statistically, T12 and T13 were equal to each other but significantly greater than all of the other treatments. For flower diameter retention, T12 had the highest flower diameter retention at $95.4 \pm 0.8\%$ meaning that treatment

T12 had retained the flowers' capitulum architecture and turgor-dependent expansion of their petal cells after 6 days in the vase. Flower diameter retention was $> 93\%$ for T13 and T11. The stem length retention had the same retention values as the flower diameter retention with T12 ($96.2 \pm 0.8\%$), T13 ($94.4 \pm 0.9\%$), and T11 ($92.4 \pm 1.0\%$) being significantly greater than all other treatments with a least significant difference of 1.38 at the $p=0.05$ level.

Malondialdehyde (MDA) is a measure of how much lipid peroxidation has occurred as well as how disrupted the membrane structure of a cell is. T12 contained the least amount of MDA (2.18 ± 0.11 nmol g⁻¹ FW) and had 58.4% less MDA than T1 (5.24 ± 0.18 nmol g⁻¹ FW). An inverse relationship was found between the amount of MDA present in T12 and its vase life ($r = -0.968$; p

< 0.01), supporting that oxidative damage to the membrane is a primary contributor to chrysanthemum senescence. Comparatively, soluble protein content in T12 ($6.48 \pm 0.20 \text{ mg g}^{-1} \text{ FW}$) was greater than that in T1 ($3.12 \pm 0.14 \text{ mg g}^{-1} \text{ FW}$) indicating a role for successful inhibition of senescence-related proteolytic enzyme activity.

Table 3. Effect of packaging materials and floral preservatives on chlorophyll content, flower diameter retention, stem length retention, malondialdehyde (MDA) content, and total soluble protein of cut chrysanthemum cv. ‘Snowball’ (pooled over two seasons, 2023–24 and 2024–25).

Treatment	Chlorophyll Content (SPAD units)	Flower Diameter Retention (%)	Stem Length Retention (%)	MDA Content ($\text{nmol g}^{-1} \text{ FW}$)	Total Soluble Protein ($\text{mg g}^{-1} \text{ FW}$)
T1 (Control)	$27.4 \pm 0.9\text{f}$	$72.6 \pm 1.6\text{f}$	$76.4 \pm 1.5\text{f}$	$5.24 \pm 0.18\text{a}$	$3.12 \pm 0.14\text{f}$
T2 (PP)	$30.8 \pm 0.9\text{e}$	$79.2 \pm 1.4\text{de}$	$80.8 \pm 1.4\text{de}$	$4.48 \pm 0.16\text{b}$	$4.06 \pm 0.16\text{e}$
T3 (LDPE)	$33.4 \pm 1.0\text{d}$	$82.8 \pm 1.3\text{cd}$	$83.6 \pm 1.3\text{cd}$	$4.12 \pm 0.16\text{bc}$	$4.52 \pm 0.17\text{d}$
T4 (CFB)	$28.6 \pm 0.9\text{ef}$	$75.4 \pm 1.5\text{ef}$	$78.2 \pm 1.5\text{ef}$	$4.86 \pm 0.17\text{ab}$	$3.48 \pm 0.15\text{f}$
T5 (mBOPP)	$29.8 \pm 0.9\text{ef}$	$77.6 \pm 1.5\text{e}$	$79.4 \pm 1.4\text{e}$	$4.62 \pm 0.16\text{b}$	$3.74 \pm 0.15\text{ef}$
T6 (Sucrose)	$35.2 \pm 1.0\text{d}$	$84.6 \pm 1.2\text{c}$	$85.8 \pm 1.2\text{c}$	$3.86 \pm 0.15\text{cd}$	$4.84 \pm 0.17\text{cd}$
T7 (Suc+8-HQC)	$38.6 \pm 1.1\text{c}$	$88.4 \pm 1.1\text{b}$	$89.2 \pm 1.1\text{b}$	$3.24 \pm 0.14\text{de}$	$5.36 \pm 0.18\text{c}$
T8 (Al_2SO_4)	$35.8 \pm 1.0\text{d}$	$85.2 \pm 1.2\text{c}$	$86.4 \pm 1.2\text{c}$	$3.74 \pm 0.15\text{cd}$	$4.96 \pm 0.17\text{cd}$
T9 (AgNO_3)	$37.4 \pm 1.0\text{c}$	$87.2 \pm 1.1\text{bc}$	$88.0 \pm 1.1\text{bc}$	$3.42 \pm 0.14\text{cd}$	$5.18 \pm 0.18\text{c}$
T10 (SA)	$34.8 \pm 1.0\text{d}$	$83.8 \pm 1.2\text{c}$	$85.0 \pm 1.2\text{c}$	$3.96 \pm 0.15\text{c}$	$4.74 \pm 0.17\text{d}$
T11 (PP+Suc+8-HQC)	$41.2 \pm 1.1\text{b}$	$91.8 \pm 1.0\text{ab}$	$92.4 \pm 1.0\text{ab}$	$2.84 \pm 0.13\text{e}$	$5.82 \pm 0.19\text{b}$
T12 (LDPE+Suc+8-HQC)	$44.8 \pm 1.2\text{a}$	$95.4 \pm 0.8\text{a}$	$96.2 \pm 0.8\text{a}$	$2.18 \pm 0.11\text{f}$	$6.48 \pm 0.20\text{a}$
T13 (mBOPP+Suc+8-HQC)	$43.2 \pm 1.1\text{ab}$	$93.6 \pm 0.9\text{a}$	$94.4 \pm 0.9\text{a}$	$2.46 \pm 0.12\text{ef}$	$6.14 \pm 0.19\text{ab}$
SEm $\pm$	0.52	0.48	0.46	0.07	0.09
CD ($p = 0.05$)	1.57	1.44	1.38	0.21	0.27

FW = Fresh Weight. Values are mean $\pm$ SEm ($n = 3 \text{ replications} \times 5 \text{ stems}$). Means within a column followed by different lowercase superscripts differ significantly at $p = 0.05$ (DMRT). MDA content for T1 represents the value at termination of its vase life (day 8); all others are at day 12 for comparability.

Vase Solution Bacterial Load, Antioxidant Enzyme Activities, and Overall Quality Score

Table 4 summarizes the findings on vase solution bacterial counts, antioxidant enzyme activities and overall quality scores. By day 8, the control vase solutions (T1) had reached $68\,400 \pm 2\,840 \times 10^3 \text{ CFU mL}^{-1}$, which agrees with the rapid proliferation of gram-negative rod bacteria (mostly *Pseudomonas fluorescens* and *Enterobacter agglomerans*) that produce exopolysaccharides to physically block the xylem vessels in cut flowers. The treatments with 8-HQC (T7, T11, T12, T13) and AgNO_3 (T9) resulted in the greatest microbial suppression. T12 had only $840 \pm 42 \times 10^3 \text{ CFU mL}^{-1}$, representing an $81.5\times$ reduction compared to T1. T13 and T11 were next with bacterial counts of $1\,480$ and $2\,160 \times 10^3 \text{ CFU mL}^{-1}$.

High levels of superoxide dismutase (SOD) activities were observed for T12, T13, and T11,

with averages of $28.6 \pm 1.1 \text{ U mg}^{-1} \text{ protein}$, 27.2 ± 1.0 , and 25.8 ± 1.0 , respectively, making these three treatments significantly greater than any of the single-factor treatments. Both catalase (CAT) and peroxidase (POD) produced values that ranked the same as the SOD where T12s POD and CAT produced values of $24.2 \pm 0.9 \text{ U mg}^{-1} \text{ protein}$ and $19.8 \pm 0.8 \text{ U mg}^{-1} \text{ protein}$, respectively, compared to T1, which had 8.2 and $5.4 \text{ U mg}^{-1} \text{ protein}$, respectively. The maintenance of high antioxidant enzyme activity levels by the combined treatments is strongly correlated to low MDA levels, with an r value (correlation coefficient) of -0.942 for SOD compared to MDA, $p < 0.01$, thereby confirming the biochemical consistency of the dataset.

The Overall Quality Score (OQS) confirmed the same ranking as the individual parameter rankings. T12 received an Overall Quality Score (OQS) of 4.8 ± 0.1 indicating flowers were

completely turgid and ready for sale, whereas T1 received a score of 1.8 ± 0.1 indicating flowers had completely senesced. T13 (4.6) and T11 (4.3) also received exemplary scores for commercial use. T12 was the best overall performer for all 14 categories measured—vase life, water relations, morphological retention, biochemical quality, microbiological control, and sensory score. This is evidence of T12 as an integrated postharvest treatment.

Table 4. Effect of packaging materials and floral preservatives on vase solution bacterial count (day 8), antioxidant enzyme activities (SOD, POD, CAT) in petal tissue (day 12), and overall quality score (OQS) of cut chrysanthemum cv. ‘Snowball’ (pooled over two seasons, 2023–24 and 2024–25).

Treatment	Vase Solution Bacterial Count ($\times 10^3$ CFU mL ⁻¹) at Day 8	SOD Activity (U mg ⁻¹ protein)	POD Activity (U mg ⁻¹ protein)	CAT Activity (U mg ⁻¹ protein)	Overall Quality Score (1-5 scale)
T1 (Control)	68,400 $\pm$ 2,840a	12.4 $\pm$ 0.6e	8.2 $\pm$ 0.4f	5.4 $\pm$ 0.3f	1.8 $\pm$ 0.1f
T2 (PP)	38,200 $\pm$ 1,920bc	15.8 $\pm$ 0.7d	11.4 $\pm$ 0.5de	8.2 $\pm$ 0.4e	2.6 $\pm$ 0.1e
T3 (LDPE)	32,600 $\pm$ 1,640cd	17.2 $\pm$ 0.7cd	13.2 $\pm$ 0.5cd	9.6 $\pm$ 0.4de	2.9 $\pm$ 0.1de
T4 (CFB)	48,800 $\pm$ 2,240b	13.6 $\pm$ 0.6e	9.4 $\pm$ 0.4ef	6.8 $\pm$ 0.3ef	2.2 $\pm$ 0.1ef
T5 (mBOPP)	42,400 $\pm$ 2,120bc	14.8 $\pm$ 0.7de	10.6 $\pm$ 0.5ef	7.8 $\pm$ 0.4ef	2.4 $\pm$ 0.1ef
T6 (Sucrose)	28,400 $\pm$ 1,420d	18.6 $\pm$ 0.8c	14.8 $\pm$ 0.6c	11.2 $\pm$ 0.5cd	3.2 $\pm$ 0.1d
T7 (Suc+8-HQC)	3,840 $\pm$ 192f	22.4 $\pm$ 0.9b	18.6 $\pm$ 0.7b	14.8 $\pm$ 0.6b	3.9 $\pm$ 0.1b
T8 (Al ₂ SO ₄)	18,600 $\pm$ 940e	19.2 $\pm$ 0.8c	15.4 $\pm$ 0.6c	11.8 $\pm$ 0.5c	3.4 $\pm$ 0.1cd
T9 (AgNO ₃)	5,120 $\pm$ 258f	21.6 $\pm$ 0.9b	17.8 $\pm$ 0.7b	13.6 $\pm$ 0.6b	3.7 $\pm$ 0.1bc
T10 (SA)	24,200 $\pm$ 1,220de	18.2 $\pm$ 0.8c	14.2 $\pm$ 0.6c	10.8 $\pm$ 0.5cd	3.1 $\pm$ 0.1d
T11 (PP+Suc+8-HQC)	2,160 $\pm$ 108g	25.8 $\pm$ 1.0a	21.4 $\pm$ 0.8a	17.2 $\pm$ 0.7a	4.3 $\pm$ 0.1a
T12 (LDPE+Suc+8-HQC)	840 $\pm$ 42h	28.6 $\pm$ 1.1a	24.2 $\pm$ 0.9a	19.8 $\pm$ 0.8a	4.8 $\pm$ 0.1a
T13 (mBOPP+Suc+8-HQC)	1,480 $\pm$ 74gh	27.2 $\pm$ 1.0a	22.8 $\pm$ 0.9a	18.6 $\pm$ 0.7a	4.6 $\pm$ 0.1a
SEm $\pm$	-	0.48	0.36	0.31	0.05
CD (p = 0.05)	-	1.44	1.08	0.93	0.15

SOD = Superoxide Dismutase; POD = Peroxidase; CAT = Catalase; OQS = Overall Quality Score (1 = completely senesced, unmarketable; 5 = fully turgid, maximum commercial value). Bacterial count data could not be homogenised across treatments for CD computation due to the extreme variance ratio; treatments marked with different superscripts differ at $p = 0.05$ (DMRT on log-transformed data). SEm $\pm$ and CD are reported for enzyme activities and OQS only.

Discussion

In this study, the continual excellence of all combined treatments based on LDPE can be accounted for by considering each of the individual physiological and biochemical actions that take place when combining the two different treatments within T12. The impregnated film (LDPE) at 25 μ m thickness has a water vapour transmission rate (WVTR) of approximately 1.0 - 1.4 g m⁻² day⁻¹ at a temperature of 23 °C with a 50% relative humidity differential, which is much lower than that of both PP (3.5 - 4.2 g m⁻² day⁻¹) and corrugated fibreboard (which will have a WVTR greater than 500 g m⁻² day⁻¹). By reducing the WVTR, the LDPE will create a high humidity micro environment around the stem and petals, thereby reducing transpirational water loss (the

primary reason for fresh weight decline and turgor loss in cut flowers; Zhou *et al.*, 2022) in cut flowers. While LDPE has somewhat less thermal barrier performance than mBOPP, the fact that the CO₂ transmission rate of LDPE is slightly greater than that of mBOPP will allow continuous accumulation of respiratory CO₂ to occur in the headspace of each package, which will suppress aerobic microbial respiration and ethylene production (El-Tanany *et al.*, 2020).

A dual action mechanism is responsible for the effectiveness of 8-HQC as the antimicrobial compound in the preservative solution: (i) the vase solution's acidification to a pH 3.8–4.2 is bacteriostatic against most gram-negative xylem-blocking bacteria such as *Pseudomonas* and *Enterobacter*; and (ii) the chelating of divalent metal ions (Ca²⁺, Mg²⁺, Fe²⁺) which are necessary cofactors in bacterial metabolic enzyme systems.

This effectively deprives the bacteria of the essential cofactors they need for growth (Kaviani *et al.* 2021; Jain *et al.* 2023). The bacterial count was decreased by 81.5 times from T12 compared to T1 by day 8, which explains the high stem water uptake rates measured in T12 until day 12, preventing occlusion of xylem vessels and sustaining the connection for hydraulics between the vase solution and petal cells.

Support for the physiological findings comes from the biochemical results. There was significantly less MDA in T12 ($2.18 \text{ nmol g}^{-1} \text{ FW}$) than in T1 ($5.24 \text{ nmol g}^{-1} \text{ FW}$), which indicates reduced lipid peroxidation (a well-known indicator of senescence in membranes). This is in agreement with significantly higher antioxidant enzyme activity levels in T12. The fact that SOD, POD, and CAT activity levels were maintained at high levels in T12 and related treatments further enhances the ability of cells to scavenge ROS generated during the normal processes of ageing and in response to stressors (Li *et al.*, 2021; Gao *et al.*, 2022). The strong negative correlation between MDA levels and vase life ($r = -0.968$), along with the strong positive correlation between enzyme activity levels and vase life ($r = 0.958$ for SOD, $p < 0.01$), provide additional evidence that effective treatments to reduce oxidative stress are an important mechanism for extending the vase life of chrysanthemum flowers after harvest.

In isolation, silver nitrate (T9) was a good individual preservative; however, when applied in combination with T7 or T8, T9 did not meet the combined treatment performance levels. The effectiveness of Ag⁺ ions to block the ethylene receptor ETR1 and strong antimicrobial activity is limited when combined with carbohydrate supplementation because this restricts the overall respiratory substrate for energy that ultimately results in progressive depletion of cellular energy and accelerated senescence compared to treatments with sucrose (Darras & Terry, 2021; Mistry & Vaishnav, 2023). Another practical issue preventing smallholder producers from using T9 for preservative production is the potential for environmental and regulatory issues associated with the disposal of silver in an agricultural effluent. Salicylic acid (T10) had the lowest level of MDA for all individual preservative treatments,

which is consistent with its known ability to induce antioxidant enzymes (Farouk & Al-Amri, 2020), compared to T7 and T9, as T10 did not possess the antimicrobial activity or provide substrate supplementation that T7 and T9 offered; thus, the overall effectiveness of T10 relative to T7 and T9 was lower.

The price difference of the combined treatment T12 vs Control is about ₹2.80–3.20 per stem (including LDPE film, sugar and 8-HQC). Chrysanthemums (Premium Grade) sell for an average of ₹120–180 per bunch of 10 stems at Wholesale prices in Metropolitan markets; T12 gave us almost double the lifespan of Marketable product compared to Control. Consequently, this gives T12 an excellent cost-to-benefit relationship. The Inputs used for Treatment are commercially available and do not require any Specialised Equipment; therefore, smallholder producers can easily adopt this technology with little Capital Investment Required (Nair *et al.*, 2020).

Conclusions

The findings of this study indicate that combining packaging with 25 micrometers of LDPE (nylon low density polyethylene) with a vase solution containing 3% (weight/volume) of sucrose and 200 mg/L of 8-HQC results in a superior postharvest management plan for cut chrysanthemum cv. 'Snowball', which resulted in a vase life that increased 127% over untreated controls, or 16.8 days. This combination of treatments was also superior for all fourteen parameters examined including water relations, retention of morphological quality, maintenance of chlorophyll, markers of oxidative stress, activities of antioxidant enzymes, suppression of microbial populations, and an overall sensory rating of quality. The superior performance of T12 can be attributed to the interaction of at least four separate physiological and biochemical mechanisms: (i) the moisture barrier provided by LDPE prevents excessive water loss through transpiration; (ii) 8-HQC prevents the growth of microorganisms that clog xylem vessels and maintains hydraulic conductivity in the stem; (iii) sucrose provides respiration substrate for extended storage; and (iv) Continued activity of antioxidant enzymes reduces oxidative

senescence. The materials used in this research are all commercially available: LDPE film rolls, food grade sucrose and 8-HQD. This method may be readily implemented with the resources available at any standard farm, requiring no capital investment. T13 (mBOPP) represents a viable treatment for enhancing the thermal protection of chrysanthemums during transport within the proposed parameters (mean of 15.4 days). The scientific validation of these results demonstrates their economic viability and will aid the development of postharvest management systems for chrysanthemum production in subtropical regions of India. Future studies should look at other cultivars, cold-chain (2°C – 5°C) storage conditions, duration simulations for exporting, and the environmental fate of 8-HQD in discharge streams from flowers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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MOVING HAULERS AS URBAN MICRO-FARMS

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Abstract

With the expanding population in urban areas urban agriculture is expanding to meet demands for local food, social engagement and environmental benefits. Currently in the market we have seen lots of moving vehicles carrying useful stuffs like moving clinics, moving restaurants, in the same way one emerging concept is the utilization of moving vehicle-based micro farms which can be also be called as “moving haulers” is proposed in the present study. These vehicles can bring fresh agriculture produce directly to the consumers. Direct relation between producer and consumer is also created here. Here in this paper, we are presenting a concept about moving haulers which is a moving vehicle with farming. The technical, financial, environmental and governance aspects of constructing a moving hauler and its use in urban areas has been analyzed in this paper. We are presenting here a technical design for movable micro-farms with fresh vegetables along with the main obstacles including its economic, logistical and regulatory aspects. This concepts also enables use of cheap drip irrigation system also, other latest advanced techniques can also promoted.

Key words: Moving Hualers, Micro-farms, Urban agriculture, Farming

Introduction

Currently twenty-first century is perceiving an remarkable speed of urbanization (Cohen, 2004). With the increasing attraction toward urban life style and in search of job more than half of Indian population has moved toward cities and this figure is going to be increased in future. When population expands the demands for the food also enhances side by side, food system is facing increasing pressure to supply safe and nutritious food from this constrained land, water and energy resources (Wang, 2022). Safe supply of green vegetables is again a major constraint in the present world scenario (Spiker *et al.*, 2023). As the pressure of demands increase money minded people start using adulteration and other ways for production and selling. For example, milk and paneer adulteration is a major issue we are witnessing

presently. Long distance supply chains are also facing several vulnerabilities (Wagner, & Bode, 2006). With these challenges the interest in urban agriculture (UA) have increased and this concept of farming on moving vehicle and supplying fresh vegetables from the vehicle can be used as a resilient strategy to localize food production with shortening supply chains and connecting producer and consumer directly. Urban agriculture gaining attention now a days, and recent innovations in technology and design have helped a lot in improving urban agriculture (Orsini *et al.*, 2013). We have lots of low-input system like community gardens, allotments and rooftop farms which give more importance to social and environmental benefits and at the same time we have high-tech solutions like vertical farms, climate-controlled greenhouses and hydroponic or aeroponic units

that help in maximizing yield. Together, these systems highlight the range of possibilities for producing food closer to where people live. This concept of moving haulers which is mobile micro-farms represent an innovative and unexplored category. Solar panel or electric based moving haulers can also be made which will be save energy also. This novel concept of bringing the farm to the people rather than asking people to travel to distant farms offers several unique benefits in terms of flexibility, accessibility and visibility. Now if we talk about urban plots they are extremely expensive and scarce, so this concept will promote expansion of urban agriculture. Mobile system will help in overcoming these barriers by making land as not fixed requirement. Till now this concept is little explored and studied, there is no review of literature on this concept. There is a need to study on its economical and practical utility aspect. As such, key questions remain unanswered. What is the technical feasibility of maintaining stable growing conditions while on the move? Are the economics favorable compared to stationary systems, considering costs of retrofitting vehicles, energy demands, and transport logistics? What are the environmental trade-offs, including emissions from mobility and energy use, compared to potential savings in distribution? And importantly, what governance and regulatory frameworks are needed to manage health, safety, road use, and land-use permissions for these units?

This paper has been proposing the moving hauler concept of promoting micro-farm unit and addressing the gaps by collecting insights from adjacent literatures about container farms, urban food trucks, peri-urban agriculture and hydroponic technologies to create a conceptual foundation for studying moving haulers. Additionally, we explore three major questions: What is the technical feasibility, economic viability, and social/environmental impacts of moving haulers? How do they are comparable to stationary urban farming models in various terms, What governance, safety, and scaling issues must be addressed for them to play a meaningful role in future urban food systems? By covering these aspects, we aim to set a research agenda and highlight the potential role of moving

haulers as a flexible, community-centered innovation for promoting urban agriculture in more sustainable way.

Definition and Concept

Moving haulers or mobile micro-farms can be defined as self-contained or semi-autonomous agricultural units that are mounted on movable platforms. These platforms may include light commercial trucks, trailers, converted vans, or shipping containers fitted with wheels or chassis. The defining characteristic is their ability to be physically transported across urban environments, either through direct mobility (driving the unit itself) or by towing and relocating. Unlike conventional urban farms that are tied to a fixed site, moving haulers introduce flexibility of location, which can be leveraged to overcome land-use constraints in cities. Their mobility may vary in frequency from daily relocations for vending or outreach, to periodic shifts between community centers or markets, or occasional moves for special events, demonstrations, or seasonal adjustments in light and climate conditions. For the purpose of this review, the scope is carefully delineated to capture systems that share this core principle of mobility. We include units that:

- (a) are specifically designed to produce food crops or edible plants such as vegetables, herbs, or microgreens;
- (b) are intended to function primarily in urban or peri-urban contexts, where land scarcity and food accessibility challenges are most acute; and
- (c) are mobile or relocatable, meaning they can be repositioned without permanent attachment to a site.

Excluded from this scope are static systems such as rooftop farms, community gardens, and permanently sited container farms. However, we include containerized farms or modular pilot units that, while not constantly mobile, retain the potential for relocation and thus align with the broader concept of moving haulers.

Typology & existing models

A typology helps clarify design trade-offs:

1. **Fully mobile truck/van farms:** Light vehicles retrofitted with vertical racks or rolling trays for microgreens, herbs, lettuces. Pros: high accessibility, low footprint; cons: limited volume, stability and regulatory constraints.

2. **Trailer/container farms on chassis:** ISO containers or custom trailers with hydroponic systems and environmental controls. Pros: larger growing area, can be towed to sites; cons: weight, permitting for road transport.
3. **Modular pop-up micro-farms:** Foldable or modular units assembled at sites from transported components. Pros: flexible footprint; cons: labor/time to assemble.
4. **Semi-mobile plug-in units:** Units are moved infrequently and plugged into local utilities when placed (e.g., markets, schools). Pros: can use local grid water/power; cons: need site agreements.

Existing analogues: container farms (static or relocatable), “truck farms” offering produce at markets, mobile gardens used for education, and modular vertical farming pilots. Empirical literature on truly mobile, production-focused haulers is sparse—most evidence must be inferred from adjacent systems.

Technical & agronomic considerations

Design must reconcile mobility with crop needs.

Growing system

- **Soil vs soilless:** Soilless (hydroponic/aeroponic) systems reduce weight and soil mess, improve water use efficiency and ease cleaning advantageous for mobility. Soil systems support wider crop range but are heavier and require more containment.
- **Crop selection:** Fast-turnaround, high-value greens (microgreens, herbs, salad leaves), mushrooms, edible flowers and certain fruiting vines (with appropriate light) are best suited. Staple crops are impractical at mobile scales.

Light & climate control

- LED lighting provides spectrally tuned lighting but consumes power. Design must balance light intensity, photoperiod and energy economics. Passive insulation, reflective interior surfaces, and small climate zones reduce energy loads.

Water & nutrient management

- Closed-loop hydroponic systems minimize water use. Onboard tanks vs local hookups: tanks allow independence but add weight; hookups require site agreements.

Power & energy

- Solar panels plus battery storage can offset grid dependency but may not fully meet loads (especially for heating/cooling and lighting). Hybrid systems (grid + renewables) are pragmatic.

Structural & mobility constraints

- Roadworthiness, weight distribution, secure mounting of racks, vibration/tilt impacts on plants and plumbing must be engineered. Regulatory dimensions include vehicle class, permits for oversized loads, and parking/occupancy rules.

Biosecurity & hygiene

- Containment prevents pests and pathogens; wastewater treatment and compost handling must meet health standards if onsite processing occurs.

Economic Viability & Business Models

The economic sustainability of moving haulers as urban micro-farms depends on balancing costs of design, operation, and maintenance with the revenues generated from diverse market channels. Unlike conventional stationary farms, these systems require both agricultural and transportation-related investments, making their cost structures unique. Cost considerations begin with vehicle acquisition and retrofit. Purchasing a truck, trailer, or container and modifying it into a functional farming unit involves significant upfront capital. This includes installing hydroponic or soil-based growing systems, climate control mechanisms, LED lighting, irrigation, and water storage. In addition, costs are incurred for seeds and bio-inputs, energy and water consumption, and routine maintenance of both farming equipment and the vehicle itself. Urban regulations may require permits, food safety

certifications, and insurance, further adding to the financial outlay. The ongoing need for labor—for crop management, harvesting, transport, and sales—is a recurring expense and often the largest operational cost. Automation, such as remote monitoring and climate control, can reduce labor intensity but requires higher capital investment.

On the revenue side, multiple business models can be deployed to diversify income streams:

1. **Direct-to-consumer sales:** Moving haulers can sell fresh produce directly in local markets, at roadside stands, or within neighborhoods. Because produce is harvested and sold on the same day, it can command premium prices. The hyperlocal branding of food grown “just outside your door” appeals strongly to urban consumers seeking freshness, transparency, and reduced food miles.
2. **Subscription and community-supported agriculture (CSA) models:** Customers may subscribe to weekly or monthly produce boxes, ensuring stable income for operators while reducing marketing and transaction costs. The mobile farm can deliver produce directly to households or community collection points.
3. **Farm-to-institution supply:** Schools, hospitals, and restaurants increasingly seek sustainable and traceable produce. Moving haulers can build long-term contracts with such institutions, providing a reliable source of fresh vegetables or herbs.
4. **Service-oriented models:** Beyond food production, moving haulers can function as educational platforms, mobile classrooms for sustainability training, or interactive exhibits at events, festivals, and corporate workshops. Corporate sponsorship or partnerships with NGOs can fund such initiatives, making them viable even when crop sales alone are insufficient.
5. **Distributed micro-network model:** A fleet of moving haulers coordinated across a city can cover multiple neighborhoods on rotation, sharing logistics such as seed sourcing, harvesting, and distribution. Centralized hubs for washing, packaging, and storage create economies of scale, improving profitability across the network.

Economic resilience for these systems hinges on several factors

- **Utilization rate:** Units that operate daily or on consistent schedules are more likely to recover high upfront costs. Idle vehicles represent sunk capital.

- **Yield per cycle and turnover rate:** Short-cycle crops such as microgreens allow multiple harvests per month, improving revenue flow.
- **Premium pricing:** Success depends on capturing higher prices through freshness, novelty, or sustainability branding, as mobile farms generally cannot compete with wholesale commodity prices.
- **Labor efficiency:** Skilled labor is critical, but costs can erode margins. Partial automation of irrigation, nutrient dosing, and climate control can reduce labor demand, though at higher capital investment.

Environmental & social impacts

Environmental

- **Pros:** Reduced food miles for last-mile distribution, potential for lower water use (hydroponics), and urban greening benefits.
- **Cons:** Energy intensity for lighting/heating and emissions from transport (unless electric). Lifecycle impacts must be evaluated (LCA).

Social

- Enhances access to fresh produce in food-insecure neighborhoods, offers educational opportunities, and creates visible green infrastructure. Mobile farms can foster community engagement and awareness about food systems.

Challenges & Limitations

Despite the promise of moving haulers as flexible and visible forms of urban agriculture, their widespread adoption faces a number of obstacles. These challenges stem from the inherent constraints of scale, energy intensity, operational logistics, regulatory frameworks, and the physical durability of mobile infrastructure. Understanding these limitations is critical for designing feasible business models, avoiding unintended consequences, and guiding supportive policies.

Research Gaps & Future Directions

For moving haulers to progress from experimental ideas to mainstream urban food strategies, several research gaps must be addressed.

1. Empirical trials Rigorous trials are needed to compare yields, input use, and costs between mobile and stationary farms across diverse climates. Such studies would provide evidence for policymakers and investors about the real performance of mobile systems.
2. Lifecycle assessments (LCA) Quantitative analyses should evaluate carbon emissions, water footprints, and resource trade-offs of mobile systems relative to conventional food supply chains. Without such data, sustainability claims remain speculative.
3. Operational research Studies on route optimization, scheduling, fleet coordination, and maintenance economics are critical for scaling. Lessons from logistics industries could inform how mobile farms operate efficiently in complex urban networks.
4. Regulatory mapping Systematic analysis of municipal policies is needed to identify barriers and opportunities for mobile farms. Clear guidelines for permits, health inspections, and road safety could ease adoption and reduce legal uncertainty.
5. Social science research Investigations into consumer acceptance, willingness to pay, and dietary impacts will clarify demand potential. Studies should also address equity concerns—who benefits, who is excluded, and how distribution can be made more just.
6. Design innovation Engineering research should focus on lightweight materials, vibration-resistant structures, and energy-efficient climate control. Integrating renewable energy more effectively and exploring novel crop varieties suited to mobility are promising directions.

Conclusions

Moving haulers, or mobile micro-farms, represent a novel extension of urban agriculture that combines food production with mobility and adaptability. By situating farms within vehicles or relocatable units, this model introduces a flexible solution to the persistent challenges of urban land scarcity and food accessibility. Unlike fixed systems such as rooftop gardens or vertical warehouses, mobile farms can travel directly to neighborhoods, schools, and community hubs, ensuring that fresh produce and agricultural experiences are brought to the people rather than

requiring people to seek them out. This portability also makes moving haulers powerful educational and outreach tools, capable of raising awareness about nutrition, sustainability, and local food systems. However, realizing this vision is far from straightforward. Mobile farms face significant engineering and operational hurdles, including energy demands, climate control, and durability of equipment under frequent relocation. Economically, they must justify high initial investments and ongoing costs while competing with conventional food supply chains. Regulatory frameworks remain unclear, with overlapping rules around vehicles, food safety, and urban land use, creating uncertainty for operators. Importantly, questions of equity must be addressed to ensure that mobile farms serve marginalized communities rather than only catering to affluent consumers or functioning as novelty attractions. Despite these challenges, moving haulers should not be dismissed. When embedded in wider urban food strategies, supported by pilot programs, and backed by interdisciplinary research and inclusive policies, they could form part of a resilient, community-centered urban food ecosystem—helping cities become greener, healthier, and more self-reliant.

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HARNESSING MODERN BIOTECHNOLOGICAL TOOLS FOR SUSTAINABLE AGRICULTURE AND FOOD SECURITY: A REVIEW OF GENOMIC, MICROBIAL AND CELLULAR APPROACHES

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Abstract

Feeding a global population expected to reach nearly ten billion by 2050 while maintaining soil, water and biodiversity and adapting to a changing climate is one of the key challenges of modern agriculture. Traditional high-input farming has increased output but at tremendous environmental and natural resource costs, making the move toward sustainable, resource-efficient systems an absolute priority. Biotechnology today offers broad based tools for assisting this transition by increasing productivity lessening the dependency on traditional agricultural chemicals. This report presents a synthesis of recent advances (2020-2026) across the full range of contemporary technologies available for modern agriculture: genomic and breeding technologies, such as reference genomes, pangenomes, super-pangenomes, molecular marker-assisted selection, genomic selection, speed breeding, doubled haploid technology, synthetic apomixis, facilitate the fast and precise development of high-yielding stress-tolerant biofortified crop varieties (Naqvi *et al.*, 2022; Tay Fernandez *et al.*, 2022; Qu *et al.*, 2024). The opportunity to edit DNA without transgenes (foreign DNA) or to produce higher-quality or more nutritious foods by using tools like the base editing system, prime editing system, multiplex editing, and epigenetic (i.e., gene regulation) editing (Tian *et al.*, 2025; Kumar *et al.*, 2022) is made possible through the use of genome-editing technologies. Using RNA interference (RNAi) by utilizing an RNAi spray application method and nanocarrier technology provides farmers with viable options for producing crops with less application than chemical pesticides (Chen *et al.*, 2025; Qiao *et al.*, 2023). The large amount of genomic data generated using artificial intelligence (AI), machine learning and high-throughput phenomic analysis provides methods for moving from these data to predictive data-based breeding pathway models (Eftekhari *et al.*, 2024; Cheng and Wang, 2024). In addition, probably the most eco-friendly solutions to improve soil fertility and manage pests in an environmentally sustainable manner are through the use of microbial (bacteria and fungi) inoculants and plant-growth-promoting rhizobacteria (Shahwar *et al.*, 2023; Alzate Zuluaga *et al.*, 2024), as well as the use of tissue culture/plant cell culture methods for rapidly and continuously propagating pest- and disease-resistant elite germplasm (Hamdan *et al.*, 2023; Duta-Cornescu *et al.*, 2023). The integration of all of these technologies will create resilient, productive, and environmentally sustainable food production systems; however, the potential benefits should be balanced with responsible regulations, biosafety, and equitable access to agricultural resources for smallholder farmers (Wang *et al.*, 2025; Raza *et al.*, 2025). Thus, the intended position of biotechnology in the sustainable food production and food security movement is as a key enabler, and not as a singular solution.

Keywords: sustainable agriculture; agricultural biotechnology; CRISPR/Cas genome editing; base and prime editing; pangenomics; spray-induced gene silencing; nanotechnology; artificial intelligence; biofertilizers; speed breeding; synthetic apomixis; food security.

Introduction

Sustainable agriculture is intended to meet the food, feed, fiber, and fuel needs of today without compromising the ability of future generations to do the same through three primary objectives of sustainable agriculture. They are; a healthy environment, economic viability, and social responsibility. Industrial, input-intensive agriculture has resulted in very high increases in production, but also has an especially high reliance on synthetic fertilizers and pesticides, as well as on fossil fuels for energy; these agricultural practices are contributing to soil degradation, water pollution, increased greenhouse gases, and the loss of biodiversity. With climate change, the reduction of arable land and a growing global population expected to be close to 10 billion by 2050, one of the major focuses of research in agriculture is now to produce a food system that is far more resource-efficient and therefore more resilient. (Raza, 2025).

Biotechnology in farming is a major step forward in producing more food with less effect on the environment. Biotechnology can make farming more productive by allowing farmers to carefully move and change desirable characteristics in seeds, create more productive soil microorganisms, and create large amounts of top-quality seeds that are free from disease. Use of biotechnology can help satisfy both the need to produce food and the need to care for the environment (Naqvi *et al.*, 2022; Hamdan *et al.*, 2023). However, biotechnology will not fix the socio-economic conditions that cause farmers to live in poverty. In addition, the successful use of biotechnology requires that other parts of the farm management system (e.g., technology, law, and equality) be in place. This paper will look at advances in three areas that complement each other—genomics and breeding, microbial

products, and plant tissue cultures—to see how the application of all three will promote sustainable agricultural production and food security.

Genomic and Breeding Technologies for Crop Improvement

The evolution of recombinant DNA technology and genomics has significantly expedited crop improvement methods. Prior to recombinant DNA technology/Genomic sequencing technologies, crop improvement through crop selection and conditional selection (i.e., selective breeding), typically took at least 10 years from start to completion of new cultivars. Today, reference genomes and pangenome sequencing have provided breeders with increased molecular target accessibility for favourable allele mobilization compared to traditional breeding methods. In addition to supplying molecular markers to ensure germplasm is accurately classified, linkage mapped, selected using marker assisted selection methods and/or combined through gene pyramiding, molecular selection methodologies have become extremely valuable for predicting breeding values of complex traits (i.e., yield or abiotic-stressed tolerance) that were considered to be low to moderate heritable (Bohra *et al.*, 2020; Budhlakoti *et al.*, 2022).

The use of fast breeding and high-throughput phenotyping together with these technologies will shorten breeding cycles and increase genetic gain for new climate-smart cultivars (Naqvi *et al.*, 2022). Genomic editing using CRISPR/Cas and related technologies has completely changed how plants are bred; now, specific modifications can be made to multiple genes at once that control yield, disease resistance, tolerance of abiotic stress, and nutrition (Hamdan *et al.*, 2023). Many recent reviews have indicated that the CRISPR/Cas system may help to improve photosynthetic, nitrogen use, and biofortification efficiencies but also come with possible off-target effects, challenges regarding delivery and regulatory approval, and when combined with synthetic biology, nanotechnology, and machine learning may provide new opportunities for breeding resilient, high-yielding crops (Hamdan *et al.*, 2023; Raza *et al.*, 2025).

Microbial Inoculants as Biofertilizers and Biopesticides

Biotechnology has made an important impact on sustainable development by helping to decrease the amount of chemical additives used in farming; this has allowed farms to become even more productive. One of the ways that biotechnology has helped make farming more efficient and environmentally friendly has been through the introduction of microbial inoculants. Microbial inoculants are specialized formulations that contain live microbes (bacteria and/or fungi) which provide benefits to crops as biofertilizers or biopesticides. Plant-growing promoting rhizobacteria (PGPR) promote the growth of crops both directly (biological nitrogen fixation, release of certain nutrients in soil, production of certain chemical compounds, etc.) and indirectly (e.g., inducing resistance to disease/pathogens). (Shahwar *et al.*, 2023).

Inoculants improve nutrient acquisition and increase biological productivity in soil to a large extent, thus decreasing the dependence on synthetic fertilisers and reducing the amount of nutrients that leave the soil in run-off. This contributes to the development of more sustainable agricultural systems as well as improved soil quality. In addition to this excellent progress in biological agriculture, the continuing evolution of omics is helping to design synthetic microbial communities (SynComs) that can provide multiple beneficial traits for plants through greater efficiencies in the acquisition of many different nutrients at the same time (Alzate Zuluaga *et al.*, 2024). These bioproducts have great potential to improve plant growth and yields when utilised at field scale; however, further advancements in delivery systems (formulations, carrier technology), shelf life, commercialisation and the understanding of soil rhizosphere ecology will be required to realise this potential (Shahwar *et al.*, 2023).

Plant Cell and Tissue Culture for Propagation and Conservation

The foundation of agricultural biotechnology still rests on plant tissue culture as a dependable method to supply genetically uniform, physiologically vigorous, and disease-free planting stock. In

addition, micropropagation allows for speedy and consistent tissue production of top-performing clones of plants so that they can be naturally grown through vegetative means; this is especially helpful in producing plants that grow slowly and at a limited production rate. Meristem culture is also frequently utilized to eliminate viruses from infected crops (Duta-Cornescu *et al.*, 2023).

Creative methodologies such as anther and microspore culture, somatic embryogenesis, somaclonal variation, embryo rescue and somatic hybridisation provide genetic diversity for the development of new cultivars; additionally, cell and hairy root cultures are also beneficial for producing useful secondary metabolites for molecular farming (Hamdan *et al.*, 2023). The ability to cryopreserve cells, tissues, and organs in liquid nitrogen provides the means for long-term preservation of germplasm, especially for species that are vegetatively propagated; as well as for other threatened or rare species. However, somaclonal variability may be a resultant consequence of in vitro processes, therefore molecular markers are often used as verification mechanisms for the genetic fidelity/trueness-to-type of micropropagated plants; this helps ensure the integrity of clonal propagation programmes (Duta-Cornescu *et al.*, 2023).

Next-Generation Genome Editing: Base, Prime and Multiplex Editing

Although CRISPR/Cas9 knockouts are still widely used as a primary method for altering crop genomes, additional tools are now being developed to produce much more accurate modifications without the use of foreign DNA. The technique of base editing can convert one type of DNA base directly into another without causing double-stranded breakage and thus allows for creating very precise single nucleotide mutations which adjust the performance of agricultural plants (Ercolano & Wang, 2023). Prime editing is a versatile 'find-and-replace' technique that uses the enzyme Cas9 nickase and a reverse transcriptase which is guided by a prime-editing guide RNA to insert all twelve types of base swaps and small deletions and insertions; and it is being refined to eliminate the low and variable efficiencies that previously limited its application to plant tissue (Tain *Et Al* 2025).

Multiplex editing, or editing multiple homoeologous copies or multiple genes simultaneously, can be especially helpful for polyploidy crops (like wheat), while CRISPR activation and CRISPR interference (CRISPRa/CRISPRi), and epigenome editing enable fine-tuning of gene expression rather than total loss of gene function. A particularly important area of application for multiplex, CRISPRa/CRISPRi, and epigenome editing is biofortification; using multiplex, CRISPR-based editing to increase the levels of nutrients such as provitamin A, iron, zinc, protein, healthy lipids, and other micronutrients in staple food crops provide a precise, non-transgenic method of improving people's access to staple foods, while combating "hidden hunger" and micronutrient malnutrition. In the same vein, it is anticipated that engineered micro-organisms will be used to improve biological nitrogen fixation and reduce reliance on synthetic fertilizers. Continued attention to minimizing potential off-target effects, maximizing delivery efficiency, and establishing clear, science-based regulations will impact the rate at which farmers are able to use these advances.

RNA Interference, Spray-Induced Gene Silencing and Nanotechnology

Emerging technologies based on RNA are a powerful, sequence-specific and environmentally friendly alternative to traditional agrochemical applications. Spray induced gene silencing (SIGS) involves applying double stranded RNA (dsRNA) topically to a plant to induce the gene silencing of essential genes in pest insects, fungi, and viruses through the process of RNA interference (RNAi) since RNAi tolerates mismatches between target sequences, causing experiences that exhibit resistance to multiple targets through use of traditional single-site fungicides (Chen *et al.*, 2025). In 2023, regulatory authorities approved the first commercially available sprayable dsRNA biopesticide entailing a significant change in the industry, resulting in rapid increases in both academic and industry research (Chen *et al.*, 2025).

A principal limitation of SIGS is that naked RNA rapidly degrades in the environment. The use of nanotechnology provides a simple solution for

this issue: artificial nanovesicles, layered double hydroxides, and other nanocarriers prevent dsRNA from degrading or being absorbed into the environment, enabling them to remain active longer within the plant cell compared to naked RNA while enhancing uptake into plant cells, thereby greatly increasing the length of time that the dsRNA will be able to provide protection to the plant (Qiao *et al.*, 2023). The application of nano-formulated products supports the development of nano-fertilizers and nano-pesticides that will improve input-use efficiencies and reduce off-target losses, and will also provide opportunities for combining with PGPR through advanced agro-biofortification (Kumar *et al.*, 2022). The combination of RNAi and nanocarriers, as well as synthetic biology designed delivery systems is leading to the creation of a new generation of targeted, low-residue, crop protection products that contribute to achieving sustainability goals (Chen *et al.*, 2025).

Artificial Intelligence, Phenomics and Data-Driven Breeding

With the large amounts of genomic, transcriptomic, metabolomic and imaging data produced today by modern biotechnology, computational analyses of these data sets have become a distinct field of study. Artificial intelligence (AI) and machine learning (ML) (including deep learning architectures such as Convolutional Neural Networks) are now being used to decode complex multi-omic data sets, predict the breeding values of plant varieties, identify stress-responsive genes, and automatically detect plant diseases so that high-yielding, stress-tolerant varieties can be developed more quickly (Eftekhari *et al.*, 2024).

Crop phenotyping represents the biggest obstacle to attempting to improve crops, with high throughput, image-based approaches such as unmanned aerial vehicles, hyperspectral and thermal images, and LiDAR, being used to collect trait data non-destructively at scale (Cheng & Wang, 2024). ML-based genome prediction using phenomic platforms reduces the genotype-phenotype gap and improves selection for complex traits like yield and abiotic-stress tolerance (Cheng & Wang, 2024). In combination with synthetic biology through rational design of

metabolic pathways and regulatory circuits, these emerging technologies are leading to the next generation of crop improvement that will be based on genomic intelligent design (Eftekhari *et al.*, 2024).

Pangenomics, Super-Pangenomes and Structural Variation

The genetic diversity of a crop species is only represented by the genome sequence of a single reference genome. Due to this fact, pangenomes have emerged as the new genomic reference system for crops (Tay Fernandez *et al.*, 2022) and they consist of the core gene content present in all populations along with the dispensable gene content found in only certain populations. Furthermore, graph-based pangenomes show much greater amounts of presence/absence and structural variant (SVs) than are available from single assemblies and many of these SVs are associated with resistance to diseases and tolerance to abiotic stressors such as temperature in pearl millet, as well as with other stressors in cereal crops (Tay Fernandez *et al.*, 2022).

Recently, the concept of the super-pangenome was added, which allows for the integration of cultivated and wild relatives on a genus-wide scale so that all alleles from a species may be represented, thus reducing the reference bias and revealing rarer, adaptive traits (Tay Fernandez *et al.*, 2022). The combination of pangenome and super-pangenome databases with genomic resources (GWAS, QTL mapping, genomic selection, and genome editing) accelerates the identification and deployment of preferred haplotypes, thereby accelerating the development of stress-resistant, high-yield crops, and incorporating previously underutilized or orphan crops into genomics (Tay Fernandez *et al.*, 2022; Bohra *et al.*, 2020)

Speed Breeding, Doubled Haploids and Synthetic Apomixis

Improving selection precision and reducing the reproductive cycle length are both significant objectives. The ability to generate several generations of plants each year, through the use of speed breeding, lights, and controlled conditions to

reduce the time it takes to produce new crops, is made possible by combining genomics selection and genome editing. (Naqvi *et al.* 2022) The production of completely homozygous (inbred) lines by means of doubled haploid (DH) technology provides a means of producing a crop from just one cycle of inbreeding rather than many cycles (Qu *et al.* 2024).

Synthetic apomixis is the method of creating a well-defined, unrepeatable hybrid vigor by using cloning technology to reproduce clonal seeds that can perpetuate the benefits associated with the heterosis of hybrid crops through clonal propagation without repeated hybridisation. Recent work with the MiMe (mitosis-instead-of-meiosis) system has demonstrated an end-to-end approach for creating clonal seeds in hybrid rice by combining MiMe with the ectopic activation of parthenogenesis genes using CRISPR/Cas technology (Qu *et al.*, 2024). Other technologies including haploid inducers and gene editing make it possible the fast & easy introduction of new traits into hybrid crops and when combined with other innovative techniques such as haploid induction & apomixis, the use gene editing will significantly shift breeding toward precision breeding & de novo domestication (Qu *et al.*, 2024) through whole-plant clonal propagation from single cells.

Integrated Biotechnology for Sustainable Production

The most significant effect comes not from an individual technique, but by combining several together. Genomic Selection and Genome Editing identify and put in new alleles with a better fit to the environment; Developing Disease-Free Plant Material is done through rapid multiplication using tissue culture; Using Microbial Inoculants will help us use less chemical inputs to express this genetic potential when we grow crops. Adding these things together with High-throughput Phenotyping, Multi-omics, Nanotechnology and Machine Learning, you will have created a data-driven pipeline to develop Climate-Resilient Cultivars and to manage them sustainably (Raza *et al.*, 2025; Naqvi *et al.*, 2022).

In addition to providing measurable environmental benefits, the integration of herbicide and pest resistant crops helps to reduce tillage and pesticide use, which minimizes carbon emissions and prevents soil erosion. Furthermore, biofertilizers help to reduce the need for synthetic fertilizers and their associated nutrient losses (Alzate Zuluaga *et al.*, 2024; Shahwar *et al.*, 2023). The global agricultural biotechnology market is expanding rapidly as demand for more sustainable and efficient methods increases. As a result, countries in Asia and around the world are utilizing marker-assisted breeding, tissue culture and genome editing methods to create crops that are resistant to disease and/or tolerant to abiotic stress (Hamdan *et al.*, 2023).

Challenges and Future Perspectives

Despite the wider promises, there are considerable constraints on agricultural biotechnology. The full potential of modern tools for generating forms has not yet been realized by resource-poor farmers, where access to land, capital, markets, and high-quality seeds often weighs as much in importance as the technology itself. Biosafety, regulatory transparency, intellectual property arrangements, and public acceptance remain critical for responsible deployment, while the development, testing, and release of biotech products must continue to be optimized in a support of innovation-sensitive manner, thereby considering considerations for human well-being and environmental safety (Hamdan *et al.*, 2023; Raza *et al.*, 2025).

Future advancements will hinge upon linking genomics, microbes and cells together with appropriate agronomic practices and fair distribution systems; therefore allowing for genetic advancement to be realised in terms of the actual benefit to a farmer's field. Strengthening both varietal release and seed distribution systems, as well as investing in small farmer specific traits and sustainable practices are very important to successfully utilize biotechnology for the production of healthy, nutritious food; conserving natural resources; preserving rural livelihoods; and improving food security in India and throughout the world (Raza *et al.*, 2025).

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PROFITABLE MODEL OF GRASSLAND FOR CARBON STOCK AND CO₂ MITIGATION POTENTIAL OF WHEAT (*TRITICUM AESTIVUM*) AGROECOSYSTEM IN SHIVALIK TARAI REGION OF HIMALAYA

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Abstract

Unbalanced land use and unsustainable farming practices are exerting pressure on soil ecosystems, shifting ecological instability and disturbing resilience, particularly in fragile regions such as the Himalayan foothills. In these landscapes, soils play a vital role in regulating carbon cycles, nutrient turnover, and hydrological balance, but remain vulnerable to degradation under poor management regimes. The present study compared soil properties and carbon stocks in two contrasting land-use systems in Pantnagar, Uttarakhand, India: a natural grassland and a wheat-based agroecosystem. Soil samples were collected bimonthly over two consecutive years at surface and subsurface soil layers to evaluate soil pH, soil organic carbon, available nitrogen in soil, potassium content, phosphorus, bulk density, and moisture content. The findings revealed pronounced depth- and season-related differences. Soil organic carbon as well as nitrogen were consistently higher at surface layers and increased in the second year, reflecting greater organic inputs and microbial activity. Phosphorus and potassium concentrations declined with depth but were relatively elevated in the wheat system, likely due to fertilizer application. Subsurface soils displayed higher bulk density and pH values. Grassland plots contributed more organic matter through continuous biomass turnover, resulting in improved fertility and carbon sequestration compared with wheat fields. Overall, the results highlight the pivotal role of land use in regulating soil carbon and nutrient dynamics, with grasslands functioning as significant carbon sinks and supporting climate change mitigation.

Key words: Agroecology, Agroforestry, Climate Change Mitigation; Land Use Systems; Soil Organic Carbon; Uttarakhand Himalaya

Introduction

Unsustainable changes in land use have exerted pressure on natural resources including soil, water and land resources. The decline in natural resources possesses deleterious effects on biotic and abiotic components of environment. This results in unsustainable soil system, changes in climate, and degradation and disintegration of

land threats to soil biodiversity and ecosystem functions (Sánchez-Cueto *et al.*, 2025). Further, the soil characteristic (like soil depth, soil texture, soil structure, water retention etc.), directly influences the drought frequency in the Himalayan region. Consequently, this is dependent on precipitation and at great extent on the prevailing management of water resource as

well as adaptation to changing climate (Lal., *et al.*, 2025). The rainfed condition is prevailing in India largely. Thus, the availability of freshwater is continuously growing concerns which is faced a huge challenge globally in way of sustainable development (Shankhwar *et al.*, 2016). Hence, the water is essentially needed for Himalayan ecosystem as in other landscapes. Therefore, water is widely considered as natural resource but inevitable and abundance but life-sustaining commodity for human as well other life on earth (Shankhwar and Srivastava, 2015). The actual source of energy supply is sun and the radial energy receiving from sun is converted to chemical energy by plants including grasses and algae. However, grasses short life span usually annual or biennial in nature. Even, lesser lifespan the grasses are continuously adding the organic matter to the soil by death, decay and disintegration. Therefore, grasses act as central sources as well as sink of energy and mass in Terrestrial ecosystems. Before understanding climate change, the causes and consequences responsible to Climate change are needed to understand. The activities by which continuous increase in CO₂ to the atmosphere, identifying sources as well as sinks of CO₂, water vapour in the atmosphere and energy at the level of whole ecosystems is of great importance. The changes in land use practices is actual root cause prognostic role in impacting the carbon cycle widely as well as influencing regional climates through altered in water balance and its energy. Terrestrial ecosystems absorb atmospheric CO₂, and with proper management, this natural process can be enhanced to effectively reduce greenhouse gases. Due to photosynthetic activities, plants capture in CO₂, store it in their bodies, and transfer it to the soil. This is the most practically sustainable way to dissipate greenhouse gas in the air and minimise the pollution. Rising atmospheric CO₂ and other greenhouse gases are largely driven by human activities such as combustion of fossil fuel, felling of forest, factory processes, and agricultural expansion. These processes intensify the greenhouse effect and contribute directly to global warming and climate change. (e.g., Ke *et al.*, 2025; Gameiro *et al.*, 2025).

To evaluate the consequences of continuously

increasing atmospheric CO₂, it is essential to identify the extent of sources and proportionately its sinks at the ecosystem, which includes the exchanges of CO₂, water, and energy. The structural composition of vegetation helps in analysing the community dynamics (Joshi *et al.*, 2007). Terrestrial ecosystems are critical in this regard: they can act as large carbon sinks by absorbing CO₂ into vegetation biomass and ultimately soil organic carbon (SOC) pools—but this capacity is strongly modulated by land-use practices, climate, soil moisture, disturbances, and land-cover change (Wang *et al.*, 2024; Nave *et al.*, 2024).

Land use and land cover changes (LULCC) substantially alter both regional energy and water balances, thereby influencing local and regional climate, as well as the global carbon cycle (Cheng *et al.*, 2024). The changes in surface energy and water balance owing to LULCC which affect regional climates and ultimately is due to the variation in net flux of CO₂ (Houghton and Nassikas, 2017). LULCC can lead to large losses of SOC—especially when forests are converted to agriculture or urbanized land; conversely, reforestation, afforestation, or other restorative practices can increase SOC and vegetation carbon stocks (Nave *et al.*, 2024; Cheng *et al.*, 2024). The changes in LULCC by deviating from tree-based carbon sequestering LULCC (Verma *et al.*, 2017) due to the sturdy increase in industrial development (Sankararamakrishnan *et al.*, 2019) and human interference (Ingole *et al.*, 2015) resulting in depletion of SOC (Bijalwan *et al.*, 2017).

SOC is a major component of terrestrial carbon stocks (often exceeding vegetation carbon stocks), and changes in SOC pools due to land use, management, and environmental conditions can meaningfully affect atmospheric CO₂ concentrations (Kopittke *et al.*, 2024; Wang *et al.*, 2024). Beyond carbon storage, SOC is also essentially required for maintaining the soil fertility as well as productivity by holding and suppling the key nutrients (e.g. nitrogen, phosphorus, sulphur) and influences soil water retention, structure, and microbial activity. The study was initiated in two systems, wheat (*Triticum aestivum*) agroecosystem and grassland ecosystem. The present research deals with the enumeration of soil properties and stock of soil organic carbon at two selected study sites.

Materials and Methods

The present paper concludes the research on natural grassland area utilised for horse riding in GBP University of Agriculture and Technology, Udham Singh Nagar, Uttarakhand, India. The location of grassland was selected in front of the Department building of Livestock Production Management (LPM), in the University premises. However, for the study on wheat, the Norman E. Borlaug Crop Research Centre (CRC) site was chosen. The grassland site near the Department of Livestock Production Management contained a mixture of native grasses, including *Cynodon dactylon*- Doob, *Echinochloa colona*-Jungle Rice, *Dichanthium annulatum*-Marvel, and *Phragmites karka*-Tall Reed, which represent typical species of the Tarai region. The study was carried out over two consecutive years, from November 2008 to November 2010, at these Centres, at University premise on a Mollisol soil order (Monika *et al.* 2017) and silty clay in texture (Shankhwar *et al.*, 2015). The site situated in Tarai plains which elevated at 243.8 m above msl (Kumar *et al.*, 2022), (29°01'30" N, 79°30'01" E), near the University's demonstration fields at the stretch of 30 km away from the central Himalaya in the southward foothills of the Shivalik range (Shankhwar & Srivastava, 2015).

The rainfall data for the experimental period were sourced from the University's Crop Research Centre observatory. The climate at the study site divides into three seasons namely summer (in the month of March to June), monsoon (in the month of July to October), and winter (in the month of November to February) (Singh *et al.*, 2023). Recent analyses in the *Tarai* region show that mean annual rainfall has been gradually decreasing by approximately 1608 mm/year (Shankhwar *et al.*, 2015). Consequently, these climatic effects have resulted in heightened fluctuations: for example, some months now record very low precipitation (trace amounts), while others exceed 300 mm. The soils are high-input and under the traditional rice–wheat sequence (RWCS) (Monika *et al.*, 2017), which heavily depletes nutrients (Jeet *et al.*, 2014). Current studies suggest that incorporating tree-based systems (agroforestry or mixed forestry) can help maintain soil fertility (Singh *et al.*, 2023). A recent field experiment showed that wheat yields

under *Populus deltoides* based agroforestry systems vary significantly depending on spacing: wider tree spacings reduce competition and increase grain yield compared to denser spacings (Sirohi *et al.*, 2023). Another study from Uttarakhand found that integrated nutrient management under agroforestry (using trees like *Grewia optiva*) improved wheat growth (Pant *et al.*, 2024).

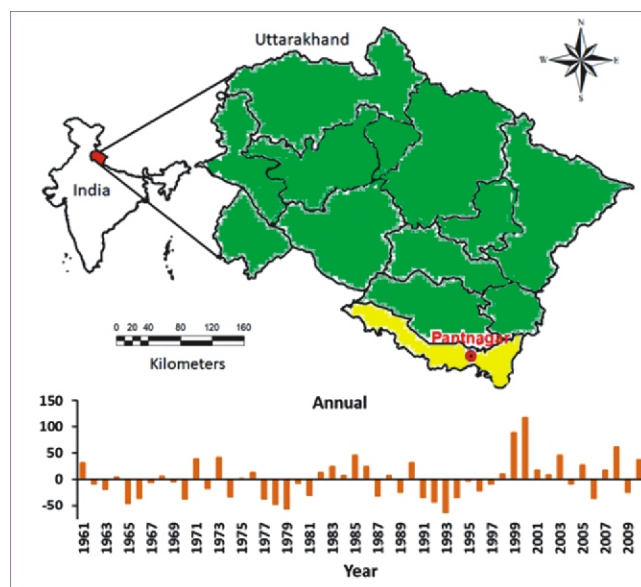


Fig. 1: Annual variation in rainfall of study area (Pantnagar)

Results

Soil characteristics: Soil samples were collected bimonthly from the plantation sites using a core sampler. At each site, eight subsamples were combined into a single composite sample to ensure representativeness. The presence of the wild grasses like Doob-*Cynodon dactylon*, Jungle Rice -*Echinochloa colona*, Marvel-*Dichanthium annulatum*, and Tall Reed- *Phragmites karka* etc. influence the soil characteristics widely. Hence for proper representation, sampling was carried out at two soil depth *i.e.* surface and subsurface (0–15 cm & 15–30 cm) soil layers, after carefully removing litter and surface vegetation. Similar sampling designs have been widely adopted in recent soil studies in plantation and agroforestry systems (Bhalave *et al.*, 2025).

For laboratory analyses, the samples were dried in shade, and coarse materials such as roots, stones, and undecomposed residues were removed. The soils were then sieved through a 2-mm mesh

and stored in sealed bags for further analyses (Lohani *et al.*, 2025). The physicochemical properties assessed included soil pH, SOC, available nitrogen (N) in soil, phosphorus (P) in soil, potassium (K) in soil, moisture content in soil, soil bulk density, and carbon stock in soil. These parameters have been identified as key indicators of soil quality under Himalayan land-use systems (Chiluveri *et al.*, 2023).

Analytical procedures followed standard protocols. Soil pH was calculated using a digital pH meter, SOC was determined by Walkley–Black modified method, and nitrogen available in soil was estimated using the Kjeldahl digestion approach. Available phosphorus and potassium were measured with spectrophotometric and flame photometric methods, respectively (Chiluveri *et al.*, 2023). Bulk density was obtained by oven-drying samples at 105°C to a constant weight and dividing soil mass by the core volume (Lohani *et al.*, 2025). Soil carbon stock was calculated as the product of SOC concentration, bulk density, and soil depth, and expressed in t ha⁻¹, following recent approaches in plantation-based studies (Bhalave *et al.*, 2025).

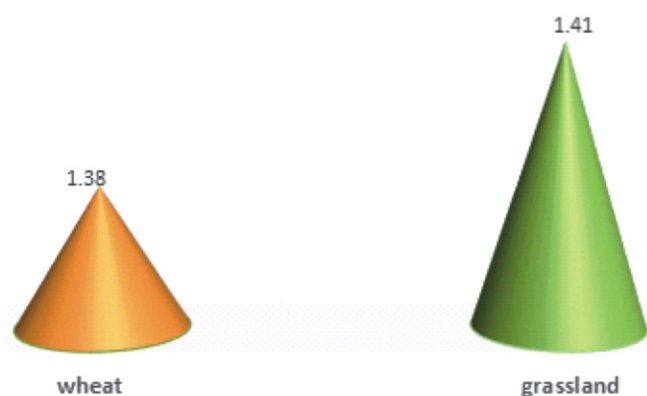


Fig. 1. Bulk density (g/cm³) in study sites

1. **Soil pH:** As pH is one of the key parameters likewise, soil pH determines the soil chemical properties as it reflects acidity, neutrality, or alkalinity, which directly affect nutrient availability and biological activity. The presence of the wild grasses like *Cynodon dactylon*, *Echinochloa colona*, *Dichanthium annulatum*, and *Phragmites karka* etc. influence the soil characteristics widely. Therefore, the temporal variation in soil pH recorded for surface and subsurface layers of soil (upto 15 cm & 15–30 cm) is presented in Figures 3 and 4. The results indicate

that soil pH generally declined in the second year across most sites, highlighting seasonal and management influences. Similar year-to-year fluctuations in soil pH under grassland-based agroecosystem have been reported in recent studies from Himalayan and subtropical regions (Sirohi *et al.*, 2023).

Several factors influence soil pH, including organic matter decomposition, fertilizer application, mineral weathering, parent material, climate variability, and land-use management (Chiluveri *et al.*, 2023). Across the two study sites, pH tended to increase with soil depth, with surface layers (0–15 cm) showing lower values compared to subsurface layers. This pattern is often attributed to the accumulation and decomposition of organic matter in surface soils, which release organic acids and lower exchangeable bases (Bhalave *et al.*, 2025). Higher soil organic carbon content in the upper layer further contributes to reduced pH values in the surface horizons (Pant *et al.*, 2024).

The observed decline in soil pH at surface depth during the study is consistent with findings from plantation and agroforestry experiments in Uttarakhand, where organic matter turnover and nutrient cycling significantly influence soil acidity (Lohani *et al.*, 2025). In contrast, slightly higher pH values at greater depths reflect reduced organic matter content and greater influence of mineral parent material (Bhalave *et al.*, 2025).

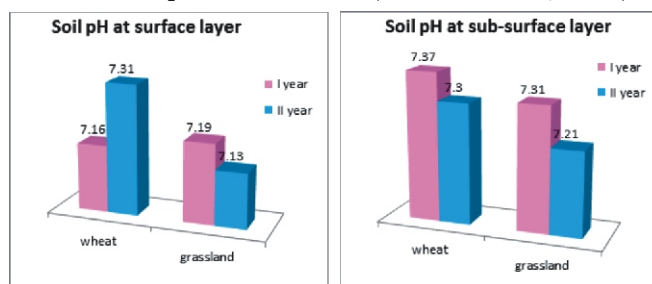


Fig. 3 & 4: Temporal variation in soil pH at surface and subsurface soil layers

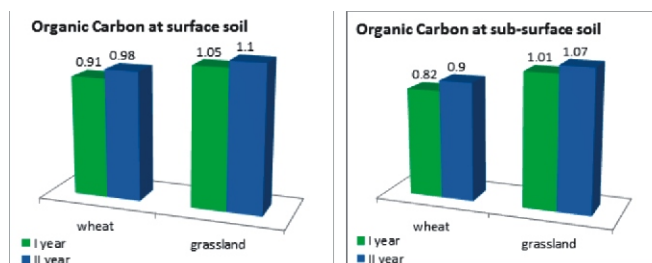


Fig. 5 & 6: Temporal variation in organic carbon at surface and subsurface soil layers

Soil organic carbon

The different sources of SOC in the cultivated land includes added crop residues, cover crops, animal manure, green manure and organic fertilizer etc. The temporal variation in SOC at surface and subsurface soil layers in both study sites namely for wheat and grassland sites have been shown in Figures 5 and 6 respectively. The figure shows that the soil organic carbon increases in second year in both sites. It was noticed that trees in association with agricultural crops increases the soil carbon status. The organic carbon through litter in trees and crop by-products increased the organic build up in their respective sites.

Soil organic carbon was consistently higher in the surface layer compared to subsurface soils. This pattern likely reflects the greater organic inputs and microbial decomposition near the surface, whereas deeper layers receive less litter and support lower microbial activity (Cheshire and Griffith, 1999). The abundance of litter available for decomposition in the surface layer makes the release of the labile compounds in the surface layer thus making it rich in terms of soil organic carbon (Post and Kwon, 2000; Xu and Xu, 2003).

The constant variation in SOC is due to the continuous changes in the climatic variables. During the monsoon season, warmer and wetter conditions accelerated decomposition, leading to higher soil carbon values compared with the drier winter months Ganuza and Almendros (2003) found that temperature was one of the main factors controlling the organic carbon level in soil. Also, the substitution of forest with pasture triggers the increase in the SOC. The SOC content in the grassland was found higher than the bamboo plantation as the decomposition of grass may be faster than the bamboo leaves. Yakimenko (1998) observed that the values for soil organic carbon were always higher in upper horizons of grass land in comparison to woodland in Russia due to rapid decomposition of grasses in the upper horizons of soils.

Available (Mineralizable) nitrogen

Nitrogen as element is abundantly present in troposphere and universally deficient in most of the soils. Owing to this the plants respond quickly to each unit of application of nitrogen to the soils.

Nitrogenous fertilizers encourage vegetative growth at above ground and provide a deep green colour to the leaves. The temporal variation in soil nitrogen at surface and subsurface soil layers in both study sites have been shown in Figures 7 and 8 respectively. The figure shows that the soil nitrogen increases in second year in both sites. In general, the available soil nitrogen content declined with the increasing soil depth in both study sites on monthly basis during study period. For all the study sites the available soil nitrogen showed increasing trend towards the end of the study period. The recorded increase in the soil nitrogen at the end of the study period in most of the vegetations implies the slow mineralization rate of nitrogen by the decomposition activities of rhizospheric microbes. Yang *et al.* (2004) observed that the soil properties are influenced due to land use change. He observed the decrease in total soil nitrogen in the rubber plantations established in tropical region of china. The temperature and moisture also account for the increasing trend of the mineralizable nitrogen in the rainy season.

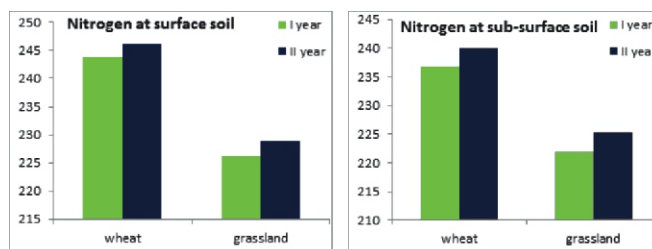


Fig. 7 & 8: Temporal variation in nitrogen at surface and subsurface soil layers

Soil Phosphorous content

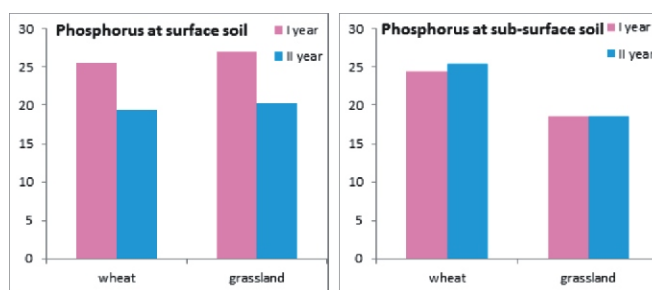


Fig. 9 & 10: Temporal variation in Phosphorus at surface and subsurface soil layers

The phosphorus major nutrient for every living cell in plant body. Thus, every vital activity of plant *e.g.* plant growth, respiration and reproduction be subject to phosphorous levels. The

temporal variation in soil phosphorus at surface and subsurface soil layers in both study sites have been shown in Figures 9 and 10 respectively. The figure shows that the soil phosphorus increases in minor quantities in second year than first year in almost both sites. Phosphorus contents were notably increased in the wheat study site, most likely due to regular fertilizer inputs that increased soil nutrient availability. Geetha and Balagopalan (2005) observed that the total phosphorous was found to be decreased with the depth in teak and eucalyptus plantations in Kerala. The observations resemble to the present study with decrease in available phosphorous in the subsurface layer of study sites. Singh *et al.* (2006) observe seasonal variation in the available phosphorous content in the agroforestry systems in Rajasthan for one year. They concluded the available phosphorus in soil to be increased after one year which is also observed in the present study.

Soil Potassium content

SOC in cultivated soils originates from crop residues, cover crops, green manures, animal manure, and organic fertilizers. Studies from the Indian Himalayas show that soils under agroforestry systems accumulate more soil organic carbon (SOC) than those under conventional agriculture, especially when trees are integrated with crops (Barman *et al.*, 2025; Maharjan *et al.*, 2024). SOC tends to increase over time, particularly in surface layers (0–15 cm), though deeper layers also show gains under long-term agroforestry (Barman *et al.*, 2025).

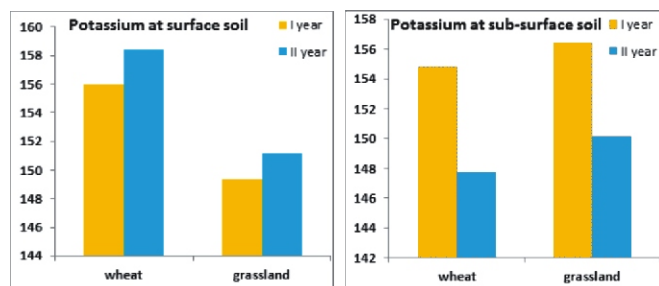


Fig. 11 & 12: Temporal variation in Potassium at surface and subsurface soil layers

Across both sites in recent studies, SOC decreases with soil depth — the topmost layer has greater accumulation because of more organic input (litter, root biomass) and more active

decomposition, whereas subsurface layers receive less of these inputs and have lower biological activity (Vikrant *et al.*, 2024; Barman *et al.*, 2025). Seasonal and climatic effects further influence SOC: during rainy seasons, moisture and warmth accelerate decomposition and organic input turnover, leading to higher SOC values compared to dry or cold seasons (Vikrant *et al.*, 2024).

Soil organic carbon stock in various study systems

Soils are recognized as major carbon sinks, and the application of nutrients such as nitrogen, phosphorus, and potassium (NPK) can enhance soil fertility while also improving carbon stabilization (Maharjan *et al.* (2024). In wheat and grassland systems, SOC stocks at surface and subsurface soil layers vary depending on litter input, root biomass, and management practices (Vikrant *et al.*, 2024). Generally, surface soils contain higher SOC levels because of the accumulation of leaf litter, crop residues, and fine roots, which decompose and form resistant humic products (Barman *et al.*, 2025). In contrast, subsurface soils often have lower SOC due to limited organic inputs and reduced microbial activity (Barman *et al.*, 2025). But the present study reveals that in the monoculture plantation like wheat agroecosystem or grassland is manipulated owing to the monoculture plantation species which effect nutrient cycling process to harness certain typical nutrients from typical soil depth. Hence, monoculture leads to deficiency of certain nutrient at certain soil depth (Shankhwar *et al.*, 2020). In contrast, with higher diversity in plants leads different root penetration at the different soil depths will be helpful to utilize the soil system without ill impacts sustainably (Shankhwar and Paliwal, 2021).

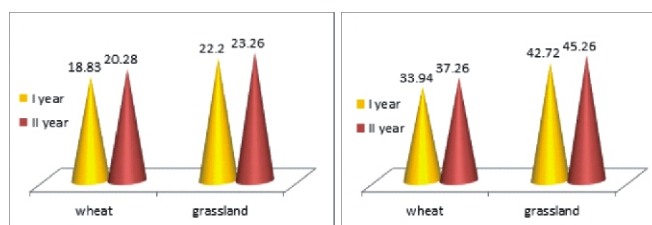


Fig. 13 & 14: Temporal variation in soil carbon stock at surface and subsurface soil layers

The application of nutrients further accelerates organic matter turnover, improves soil

fertility, and supports long-term SOC stabilization. (Maharjan *et al.*, 2024). Grassland-based systems in particular provide additional litter and root turnover compared with monocropping, which significantly enhances SOC and strengthens the role of agroforestry in carbon sequestration and climate change mitigation (Das *et al.*, 2024).

Discussion

The present study summarises the strong influence of land use changes on soil properties as well as carbon dynamics in the Himalayan *Tarai* region. The observed higher levels of organic carbon and available nitrogen in the surface soils compared to subsurface layers are consistent with findings from long-term studies in agroecosystems and grasslands, where organic matter accumulation and microbial activity are concentrated near the soil surface (Barman *et al.*, 2025; Vikrant *et al.*, 2024). The grassland ecosystem enhanced soil carbon storage, largely because of constant litter input and root cycling, which supplied organic matter to surface soils throughout the year (Das *et al.*, 2024).

The increase in SOC and nitrogen content in the second year indicates the role of seasonal biomass input and favourable climatic conditions for decomposition, especially during the monsoon. Similar patterns of temporal variation have been reported in agroforestry and plantation systems across subtropical and temperate regions, where rainfall and temperature strongly regulate microbial activity and nutrient mineralization (Maharjan *et al.*, 2024). Conversely, subsurface layers consistently showed lower nutrient content, reflecting reduced organic input and microbial biomass, a trend well documented in global soil carbon studies (Kopittke *et al.*, 2024).

Phosphorus and potassium dynamics in the wheat fields were largely shaped by external fertilizer application, which elevated their availability compared with grassland soils. However, the declining trend of these nutrients with depth underscores the need for balanced nutrient management strategies to prevent leaching losses and ensure long-term fertility (Chiluveri *et al.*, 2023). Grassland soils, despite lower phosphorus and potassium inputs,

demonstrated higher stability in organic carbon, highlighting their role in supporting soil structure and long-term nutrient cycling. Bulk density and pH trends further reveal the influence due to changes in land use and its management. The higher bulk density in subsurface soils suggests compaction and reduced porosity, which can limit root penetration and microbial activity. In contrast, surface soils under grassland conditions exhibited better structure due to organic matter accumulation, consistent with reports from Himalayan and Indo-Gangetic plains agroecosystems (Singh *et al.*, 2023). The observed decline in surface soil pH over time may be linked to organic matter decomposition and acidifying effects of litter, as also noted in plantation-based studies (Lohani *et al.*, 2025).

Taken together, the findings emphasize the critical role of grassland ecosystems in maintaining soil organic matter, regulating nutrient availability, and enhancing soil resilience under changing climatic conditions. While wheat-based agroecosystems remain central to regional food security, their sustainability can be strengthened by integrating tree-based systems, organic amendments, and diversified land use. Such practices not only improve soil fertility and carbon sequestration but also align with global climate change mitigation goals (Cheng *et al.*, 2024; Nave *et al.*, 2024).

Summary

The present study investigated the influence of changes in land use on soil quality and carbon dynamics by comparing a natural grassland ecosystem with a wheat-based agroecosystem in Pantnagar, Uttarakhand. Soils were sampled over two consecutive years at surface & subsurface soil layers and summarized for pH, organic carbon, available nitrogen, phosphorus, potassium, bulk density, and soil moisture. Results showed that surface soils consistently contained higher levels of organic carbon and nitrogen than subsurface layers, largely due to organic matter inputs and microbial activity. Grasslands exhibited greater accumulation of organic carbon compared with wheat fields, reflecting their continuous biomass turnover and litter input. Phosphorus and potassium levels were generally higher in the

wheat system, influenced by fertilizer application, while bulk density and pH tended to increase with depth. Seasonal variation was evident, with higher nutrient turnover and carbon content during the rainy season compared to winter months. The study highlights that grasslands play a critical role in carbon sequestration and soil fertility maintenance, while cultivated systems are more dependent on external nutrient inputs.

Conclusion

The comparative assessment of grassland and wheat-based ecosystems in the Tarai region of Uttarakhand demonstrates that land use practices strongly regulate soil carbon storage and nutrient availability. Grasslands, through continuous organic matter addition and root turnover, enhance soil organic carbon, improve nutrient cycling, and sustain soil fertility. In contrast, wheat systems, while productive, rely on external fertilizer inputs and exhibit lower organic carbon stocks, particularly in subsurface soils. The land use management strategies that integrates natural vegetation or grassland elements into cultivated systems could enhance soil quality, stabilize carbon stocks, and contribute to mitigation strategies of climate change. These findings reinforce the importance of sustainable land-use planning that values grasslands not only as biodiversity reservoirs but also as efficient natural sinks of atmospheric carbon dioxide.

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IN VITRO EVALUATION OF THE ANTIMICROBIAL SYNERGY OF *CURCUMA LONGA* AND *ALLIUM SATIVUM* EXTRACTS AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE CLINICAL BACTERIAL ISOLATES

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Abstract

Interest in antibacterial products from plants is revived by the ongoing increase of drug-resistant bacteria by bacteria that do not respond to antibiotics. This research looked at individual and combined doses of garlic and turmeric for their antibacterial properties to resistant bacterial strains. This trial involved numerous variables, including extraction yields, total 47368 phenolics, total flavanoids, and a qualitative phytochemical profile, both ethyl and water extracts were analyzed for their antibacterial activity using an agar diffusion assay. In addition, minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were obtained through microdilution methods according to guidelines provided by the Clinical and Laboratory Standard Institute (CLSI). The interaction between the two ethyl extracts was evaluated using the checkerboard method and determined FICI was used to determine which extract worked best together. In all cases, the three trials were replicated and reported as mean $\pm$ standard deviation with differences measured using ANOVA and Tukey's test ($p < 0.05$). Finally, both ethyl extracts of Gill and turmeric produced the lowest (~ 6.25 mg/mL) MIC for *S. aureus* as compared to both (~ 25 mg/mL) for *E. coli*. The turmeric/garlic combination was found to be synergistic against both *S. aureus* and *K. pneumoniae* (FICI = 0.38) but did not exert an effect on *E. coli* because the inhibition achieved was due to the garlic alone. These data suggest that both garlic and turmeric should be considered for their antibacterial properties, but that the turmeric/garlic combination has been shown to produce a genuine and quantifiable synergy against 2 different Gram-positive organisms and the Gram-negative organism *Klebsiella* and that there was no synergy against *E. coli*.

Keywords: Turmeric, garlic, *Curcuma longa*, *Allium sativum*, minimum inhibitory concentration, fractional inhibitory concentration index, synergy.

Introduction

Bacteria continue to inflict an enormous toll in terms of mortality and morbidity throughout the world, with the slow decline in antibiotic efficacy necessitating immediate action towards

identifying new antibacterial agents. Millions of lives are lost worldwide every year due to antimicrobial resistance and the resulting pressure placed on health care systems has brought renewed interest in finding new possible sources for antibacterial compounds

(Antimicrobial Resistance Collaborators, 2022). In this regard, both medicinal plants and culinary spices are of primary interest due to their relatively low cost, broad distribution and many years of evidence showing their medicinal uses.

Plants accumulate many different types of secondary metabolites, such as alkaloids, flavonoids, terpenoids, tannins, saponins and steroids, many of which exhibit measurable biological activities. These components are responsible for the antimicrobial, antioxidant and anti-inflammatory properties attributed to plant extracts (Khatri *et al.*, 2023). Crude solvent extracts of plant materials have been shown to inhibit a wide range of pathogens and multiple drug-resistant organisms, sometimes with effectiveness approaching that of traditional antibiotics (Tufa, 2021; Naseer, 2024).

Curcuma longa, commonly known as turmeric, is part of the ginger family (*Zingiberaceae*). Curcumin and its related compounds (*curcuminoids*) account for much of the biological activity associated with turmeric. Curcumin and curcuminoids exhibit in vitro antioxidant and antibacterial activities, including activity against *Staphylococcus aureus* strains that form biofilms and are resistant to antibiotics (Suwal *et al.*, 2021; Rattanasuk *et al.*, 2023). *Allium sativum* (garlic) is part of the amaryllis family (*Amaryllidaceae*). The primary biologically active compounds in garlic are organo sulfur compounds (primarily allicin and allyl sulfides) that act by disrupting the bacterial membrane as well as inactivating thiol-containing enzymes. This gives garlic broad-spectrum activity against both Gram-positive and Gram-negative organisms (Bhatwalkar *et al.*, 2021).

Currently, although the separate activities of the different spices have been documented individually, only a small number of studies have compared each of them with the same panel of clinical isolates and conducted thorough testing to see if the combined treatments offer any advantages.

Furthermore, claims of 'synergism' based solely on comparing the diameter of the Zones of Inhibition are not valid arguments; a valid conclusion will require that quantitative endpoints are established and that a

checkerboard analysis is completed (Su *et al.*, 2020). As such, the present study (i) characterises each of the extracts in terms of yield, total phenolic and flavonoidal content and qualitative phytochemistry; (ii) compares their antibacterial effects by conducting agar-well diffusion and also determining MICs and MBCs for each extract; (iii) evaluates the synergistic effect of turmeric and garlic against *S. aureus*, *E. coli* and *K. pneumoniae* using a Fractional Inhibitory Concentration Index.

Materials and Methods

Collection and Preparation of Plant Material

I bought fresh turmeric rhizomes and garlic bulbs from a local market in Meerut. These were authenticated as genuine turmeric and garlic. Turmeric rhizomes were peeled, washed, sliced; garlic bulbs were peeled and shade dried at room temperature. The dried materials were at a constant weight and ground into a fine powder using sterile mortar and pestle, then packed into sealed containers with no exposure to light until they were used for extraction.

Glassware Sterilisation

All glassware was washed with detergent, rinsed and sterilised in a hot-air oven at 160 °C for 1 h before use.

Preparation of Extracts and Determination of Yield

The spice powder (40 g) was macerated into 200 mL solvent of either 95% ethanol or sterile distilled water for 72 hours with frequent shaking for the two solvents. Each mixture was filtered through Whatman No. 1 filter paper and the resulting filtrate was concentrated to dry (filtrate from aqueous extracts was dried using a water bath at 40 °C) under reduced pressure using a rotary evaporator. Percent yield of the extracts was calculated by (mass of dried extract / mass of powder) x 100. To prepare stock solution of each dried extract (maximum strength of 200 mg/mL), the dried extracts were reconstituted with 10% (v/v) DMSO, sterilised by passing through a 0.22 µm filter, and then diluted in DMSO to working concentrations (100, 75, 50, and 25 mg/mL). The extracts were named as follows: TEE (Turmeric Ethanol Extract), TAE (Turmeric Aqueous

Extract), GEE (Garlic Ethanol Extract), GAE (Garlic Aqueous Extract).

Total Phenolic and Total Flavonoid Content

Total phenolic content was measured by the Folin-Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g), using a gallic acid calibration curve. Total flavonoid content was determined by the aluminium chloride colorimetric method and expressed as milligrams of quercetin equivalents per gram (mg QE/g), following procedures comparable to those used for related plant extracts (Naseer, 2024). All measurements were performed in triplicate.

Qualitative Phytochemical Screening

Alkaloids were screened (Dragendorff's reagent), saponins (persistent frothing test), phlobatannins (boil in diluted hydrochloric acid), tannins (Ferric Chloride), steroids (Acetic Anhydride-Sulphuric Acid), terpenoids in Chloroform-HCl test, flavonoids (by ammonia followed by concentrated sulphuric acid), and anthraquinones (Borntrager's test).

Test Organisms and Quality Control

Three clinical isolates each of the species *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* were acquired from a microbiology diagnostic lab and characterised by using routine biochemical identification techniques (catalase & coagulase testing for *S. aureus*; testing for indole/citrate triple sugar iron for Gram negatives). Quality Control reference strains used during testing were *S. aureus* ATCC 25923; *E. coli* ATCC 25922; *K. pneumoniae* ATCC 700603. Three bacterial isolates were all maintained on nutrient agar slants and all were standardised before the beginning of testing. As this study utilized previously isolated bacterial specimens, and no human subjects were involved in this study, no ethics and no informed consent was necessary to proceed with this work.

Agar-Well Diffusion Assay

Inocula were obtained from overnight cultures and diluted to give a 0.5 McFarland suspension (approximately 1.5×10^8 CFU/mL). Each inoculum was uniformly swabbed onto

Mueller-Hinton agar plates prepared to an approximate depth of 4 mm. Using a sterile cork borer, 8 mm diameter wells were placed into the agar plates. Then, 100 μ L of the extract for each concentration was added to the wells. Ciprofloxacin (5 μ g) served as the positive control, while 10% DMSO was used as the negative control. The plates were incubated for 24 hours at 37 °C, and the zone of inhibition (including the 8 mm well) was measured with a calibrated ruler. Each determination was performed in triplicate.

MIC and MBC by Broth Microdilution

Based on CLSI recommendations (Clinical Laboratory Standards Institute, 2022), the MIC was assessed via broth microdilution methodology in 96 well plates. Two-fold serial dilutions of each extract (0.78 -100 mg/mL) were prepared in Mueller Hinton broth and inoculated at a final concentration of approximately 5×10^5 CFU/mL. After 18 - 20 h incubation at 37 °C, the MIC was determined as the lowest extract concentration where there was no visible growth, verified using resazurin. To determine the MBC, aliquots obtained from the wells with concentrations higher than or equal to the determined MIC were sub-cultured onto Mueller Hinton agar, and the MBC was then defined as the lowest concentration where no colonies developed. A MBC/MIC ratio of < 4 suggests a bactericidal action.

Checkerboard Assay and FICI

The checkerboard microdilution method was used to measure how ethanolic extracts of garlic and turmeric would interact with each other—the FICI was calculated as $FIC = (MIC \text{ of A with B}) / (MIC \text{ of A \& B}) + (MIC \text{ of B with A}) / (MIC \text{ of B \& A})$. Results were interpreted based on the FICI values as follows: strong ($FICI = 0.5$), none or indifferent ($0.5 < FICI = 4$) and weak ($FICI > 4$) (Su *et al.*, 2020).

Statistical Analysis

The results of all trials were reported as mean $\pm$ standard deviation from three different repetitions of each experiment. One-way ANOVA with Tukey's test was used to analyse the data, and a p-value < 0.05 was defined as statistically significantly different.

Results

Extraction Yield

The percentage yields of the four extracts are given in Table 1. Garlic gave higher mass yields than turmeric, and aqueous extraction produced more crude material than ethanol, reflecting the larger contribution of water-soluble carbohydrates in the garlic bulb.

Table 1. Percentage yield (w/w) of the turmeric and garlic extracts

Extract	Solvent	Yield (% w/w)
Turmeric (TEE)	95% ethanol	11.8
Turmeric (TAE)	Distilled water	8.4
Garlic (GEE)	95% ethanol	14.2
Garlic (GAE)	Distilled water	16.5

Values are means of triplicate extractions.

Total Phenolic and Flavonoid Content

Total phenolic and flavonoid contents are summarised in Table 2. Turmeric extracts contained markedly more phenolics and flavonoids than garlic extracts, and for both spices the ethanolic extract was richer than the aqueous extract, consistent with the better solubility of curcuminoids and other polyphenols in ethanol.

Table 2. Total phenolic and total flavonoid content of the extracts

Extract	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
TEE	62.4 ± 1.8	28.7 ± 0.9
TAE	41.2 ± 1.4	18.3 ± 0.7
GEE	24.6 ± 1.1	12.4 ± 0.5
GAE	18.9 ± 0.8	9.1 ± 0.4

Values are mean ± SD ($n = 3$). GAE: gallic acid equivalents; QE: quercetin equivalents.

Qualitative Phytochemistry

The qualitative profile (Table 3) showed steroids, flavonoids, terpenoids and alkaloids in all four extracts. Anthraquinones were detected only in turmeric, while saponins and tannins were confined to garlic. Phlobatannins were absent from every preparation.

Table 3. Qualitative phytochemical screening of the extracts

Constituent	TEE	TAE	GEE	GAE
Steroids	+	+	+	+
Anthraquinones	+	+	—	—
Flavonoids	+	+	+	+
Saponins	—	—	+	+
Phlobatannins	—	—	—	—

Tannins	—	—	+	+
Terpenoids	+	+	+	+
Alkaloids	+	+	+	+

“+” = detected; “—” = not detected.

Agar-Well Diffusion: Single Extracts

The zones of inhibition produced by the turmeric extracts are shown in Table 4. The ethanolic extract was the most active against *S. aureus* (14.0 ± 0.6 mm at 100 mg/mL), followed by the aqueous extract (12.0 ± 0.5 mm). Both turmeric extracts gave a modest 8 mm zone against *K. pneumoniae* at 100 mg/mL but produced no measurable inhibition of *E. coli* at any concentration. Activity fell progressively with concentration and disappeared at 25 mg/mL.

Table 4. Zones of inhibition (mm) produced by turmeric extracts

Concentrations (mg/mL)	Staphylococcus aureus		Escherichia coli		Klebsiella pneumoniae	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
100	12.0 ± 0.5	14.0 ± 0.6	—	—	8.0 ± 0.4	8.0 ± 0.5
75	8.0 ± 0.4	9.0 ± 0.5	—	—	—	6.0 ± 0.3
50	—	6.0 ± 0.3	—	—	—	—
25	—	—	—	—	—	—

Mean ± SD ($n = 3$); zones include the 8 mm well; “—” = no inhibition. Positive control ciprofloxacin (5 µg): *S. aureus* 21.0 ± 0.8, *E. coli* 16.0 ± 0.6, *K. pneumoniae* 18.0 ± 0.7 mm. Negative control (10% DMSO): no inhibition.

Garlic extracts inhibited all three organisms (Table 5). The ethanolic extract gave 13.0 ± 0.6 mm against *S. aureus*, 11.0 ± 0.5 mm against *E. coli* and 8.0 ± 0.4 mm against *K. pneumoniae* at 100 mg/mL, with the aqueous extract slightly less active in most cases. Garlic therefore showed a broader spectrum than turmeric, retaining activity against the *E. coli* isolate that turmeric did not affect.

Table 5. Zones of inhibition (mm) produced by garlic extracts

Concentrations (mg/mL)	Staphylococcus aureus		Escherichia coli		Klebsiella pneumoniae	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
100	11.0 ± 0.5	13.0 ± 0.6	10.0 ± 0.5	11.0 ± 0.5	9.0 ± 0.4	8.0 ± 0.4
75	8.0 ± 0.4	10.0 ± 0.5	7.0 ± 0.4	8.0 ± 0.4	6.0 ± 0.3	6.0 ± 0.3
50	—	7.0 ± 0.4	—	6.0 ± 0.3	—	—
25	—	—	—	—	—	—

Mean ± SD ($n = 3$); zones include the 8 mm well; “—” = no inhibition. Controls as in Table 4.

MIC and MBC

The MIC and MBC values (Table 6) confirmed the diffusion results. The lowest MIC (6.25 mg/mL) was recorded for both ethanolic extracts against *S. aureus*, with MBC/MIC ratios of 2 indicating a bactericidal effect. Turmeric extracts had no detectable endpoint against *E. coli* (MIC and MBC > 100 mg/mL), whereas garlic inhibited *E. coli* at 12.5–25 mg/mL. The largely bactericidal MBC/MIC ratios across the susceptible organisms indicate that the extracts kill rather than merely suppress the test bacteria.

Table 6. MIC and MBC (mg/mL) of the single extracts

Extract	Staphylococcus aureus		Escherichia coli		Klebsiella pneumoniae	
	MIC	MBC	MIC	MBC	MIC	MBC
TEE	6.25	12.5	>100	>100	25	50
TAE	12.5	25	>100	>100	50	>100
GEE	6.25	12.5	12.5	25	25	50
GAE	12.5	25	25	50	25	50

Values in mg/mL; “>100” = no endpoint within the tested range (0.78–100 mg/mL). An MBC/MIC ratio = 4 indicates bactericidal action.

Checkerboard Interaction (FICI)

The checkerboard analysis of the combined ethanolic extracts is presented in Table 7. The combination was synergistic against *S. aureus* and *K. pneumoniae*, with an FICI of 0.38 for both organisms, reflecting a four- to eight-fold reduction in the effective concentration of each spice. Against *E. coli*, the FICI could not be calculated because turmeric had no measurable MIC; the garlic MIC was reduced only modestly in the mixture and the interaction was classed as indifferent, indicating that activity against *E. coli* was attributable to garlic alone.

Table 7. Checkerboard FICI for the combined ethanolic turmeric (TEE) and garlic (GEE) extracts

Organism	TEE alone / combo	GEE alone / combo	FICI	Interpretation
<i>S. aureus</i>	6.25 / 1.56	6.25 / 0.78	0.38	Synergy
<i>K. pneumoniae</i>	25 / 6.25	25 / 3.125	0.38	Synergy
<i>E. coli</i>	>100 / -	12.5 / 3.125	ND	No interaction

MIC values in mg/mL (alone / in combination). FICI = 0.5 = synergy; 0.5 < FICI = 4 = no interaction; FICI > 4 = antagonism

(Su et al., 2020). ND: not determinable because turmeric had no MIC against *E. coli*.

Combined Extract: Diffusion Across Concentrations

The zones of inhibition for the 1:1 combined extract across all concentrations are given in Table 8. Against *S. aureus* and *K. pneumoniae* the combination produced larger zones than either spice alone (for example, 16.0 ± 0.6 mm against *S. aureus* in ethanol, exceeding 14 mm for turmeric and 13 mm for garlic), consistent with the synergy detected by FICI. Against *E. coli*, however, the combined zone (9.0 ± 0.4 mm in ethanol) was smaller than that of garlic alone (11 mm), confirming that turmeric contributed nothing and that mixing simply diluted the active garlic component.

Table 8. Zones of inhibition (mm) of the combined turmeric-garlic extract

Concentration (mg/mL)	Ethanolic combination			Aqueous combination		
	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
100	16.0 ± 0.6	9.0 ± 0.4	11.0 ± 0.5	13.0 ± 0.5	8.0 ± 0.4	10.0 ± 0.5
75	12.0 ± 0.5	6.0 ± 0.3	8.0 ± 0.4	10.0 ± 0.5	6.0 ± 0.3	7.0 ± 0.4
50	9.0 ± 0.4	—	6.0 ± 0.3	—	—	—
25	—	—	—	—	—	—

Mean $\pm$ SD ($n = 3$); 1:1 (v/v) combination; zones include the 8 mm well; “—” = no inhibition.

Discussion

According to phytochemical and quantitative analyses, garlic and turmeric contain a number of bioactive metabolites that are of interest. Both garlic and turmeric have high levels of curcumin pigments, which could explain their larger amounts of phenolic and flavonoid content. In comparison to turmeric, garlic also contains saponins and tannins along with organosulfur components that match profiles typically reported in spices. Phenolics, flavonoids, and terpenoids have traditionally been thought to cause membrane damage through enzyme inhibition; whereas, tannins have typically been thought to interfere with protein synthesis in microorganisms. The above observations may provide an explanation for the activity reported herein.

Ethanollic extracts of both spices demonstrated concentration-dependent activity against both test organisms. In addition, they demonstrated greater antimicrobial activity than aqueous extracts in both diffusion and dilution assays. These differences are likely due to the ability of ethanol to more effectively extract the lipophilic curcuminoids from turmeric and the organosulfur compounds from garlic when compared to water (Bhatwalkar *et al.*, 2021; Rattanasuk *et al.*, 2023). The low MIC of the ethanollic turmeric extract against *S. aureus* (6.25 mg/mL) and the bactericidal MBC/MIC ratio support findings that curcumin-rich preparations are highly effective against this Gram-positive pathogen, including biofilm-forming strains (Suwal *et al.*, 2021).

Garlic has demonstrated antibacterial activity against *E. coli*, while turmeric has not shown any ability to kill this pathogen. The outer membrane of Gram-negative bacteria has a lipopolysaccharide (LPS) layer and specific porins that limit the entry of many hydrophobic plant components, while efflux pumps can remove those that are able to penetrate. This is an important mechanism for the literature on antimicrobial resistance (Antibiotic Resistance Collaborators, 2022). Garlic contains small and reactive organosulfur compounds, which are likely able to penetrate through the outer membrane of *E. coli*, thus resulting in a much broader range of activity for garlic extracts (Bhatwalkar *et al.*, 2021). *S. aureus* has consistently displayed the highest degree of susceptibility to both garlic and turmeric, whereas the resistance of our Gram-negative isolates to both extracts falls in line with known trends observed when screening extracts of plants (Tufa, 2021).

This point is very important in that when inferring “synergistic” activity based only on zone diameters as reported in the past, that would not be appropriate (i.e. at least a checker-board FIC index need be determined, Su *et al.*, 2020). When a checker-board FIC index was determined for the turmeric-garlic combination against both *S. aureus* and *K. pneumoniae*, the result indicated true synergy (FIC index=0.38), allowing for reductions in minimum inhibitory concentrations (MIC) of both curcumin (turmeric) and the organosulfur compounds of garlic by severalfold as

compared to each used separately. An interpretation that might be reasonable is that, due to the potential ability of the organosulfur compounds from garlic to permeabilize cell membranes, the entry of curcumin into *S. aureus*/*K. pneumoniae* would be facilitated. In contrast, with *E. coli* the addition of garlic to curcumin did not provide any benefits, as noted by the inability to define an MIC for turmeric; the inability to calculate an FIC for the combination (due to lack of meaningful zones of inhibition); and the fact that the combined zones were less than the zone for garlic alone, thereby indicating that turmeric diluted the only active agent in their combination. This type of organism-dependent synergistic effect provides more useful information than an unconditional premise of synergistic effects, thereby emphasizing the need to quantitatively characterize combination effects rather than make assumptions.

Study Limitations

Two spices and three isolates of bacteria were studied using crude extracts and the active ingredients (e.g., curcumin and allicin) were not quantified. Time kill kinetics and mechanism of action studies were not performed. The study was done in standard laboratory conditions and would need to be validated for clinical significance based on the millimolar level concentrations required. Future work should quantify the major active ingredients; include additional and multidrug-resistant isolates; and do kill timings and toxicity studies on the bacteria isolated.

Conclusion

Extracts of turmeric and garlic (in ethanol and water) exhibited a dose-response antibacterial effect against multiple bacterial strains (*Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*); the ethanol-based extracts were generally more effective, and *S. aureus* was the bacterial strain most susceptible to the effects of the extracts. The determination of minimum inhibitory and minimum bactericidal concentrations confirmed that extracts produced a largely bactericidal effect on bacteria sensitive to the extracts, with the exception of *E. coli*, for which turmeric was inactive. Checkerboard analysis

revealed that combinations of turmeric and garlic had true synergistic interactions against both *S. aureus* and *K. pneumoniae* (calculated FICI of 0.38), whereas they showed no interaction against *E. coli*. These observations support that turmeric and garlic have the potential to be used as natural antibacterial agents in susceptible bacteria, particularly when combined; however, it also clearly demonstrates that the benefits of combining them will vary depending on the bacterial species and that the potential synergism must be verified through quantitative testing rather than assumed.

Declarations

Ethical approval: The study used previously obtained bacterial isolates and did not involve human participants; ethical clearance and informed consent were therefore not required.

Conflict of interest

The authors declare no conflict of interest.

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VERTICAL FARMING OF VEGETABLES IN INDIA

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Abstract

The growth and production of crops on vertically stacked layers and vertically inclined surfaces is known as vertical farming, and it may be an efficient approach to increase food output with less area. It is necessary to construct such farms that require less land and less time to transport food produced in rural regions to urban areas in order to grow food there. The market for vertical systems is rapidly growing, and aeroponics uses comparable methods. Anyone who wants to cultivate vegetables, medicinal plants, and flower crops vertically can utilise this approach. In India, vertical farming has difficulties with mainstreaming, public awareness, farmers' involvement, technical skills, expense of operating vertical farm systems, and financial feasibility.

Keywords: Vegetables, Vertical farming, Hydroponics, Aeroponics, Aquaponics

Introduction

On Planet, only a fourth of the surface is covered by land; the remainder is covered by ocean. Due to the modest size of the arable land, which accounts for 11% of the total land area and 38% of the agricultural land. Additionally, according to forecasts, there will only be a 2% growth in agricultural area by 2040, and by 2050, groundwater levels would have drastically depleted, making it difficult to obtain water for drinking and irrigation. As a result, we need a crop production system that will enable us to satisfy the needs of the population while using less land and less natural resources. Vertical farming has the potential to reduce environmental problems since it allows for the production of more food while utilising fewer resources and less water. According to Al-Chalabi (2015), this phenomenon is gaining ground quickly in the scientific and industrial communities and has resulted in the reliable delivery of commodities to demand centres, which has reduced the need for storage and refrigeration.

India's agricultural land is constantly

moulting. In order to address the problems caused by the overpopulation, water scarcity, climate change, labour shortage, and urbanisation that is reducing the amount of arable land, newer technologies are being developed. In 1915, Gilbert Ellis Bailey published a book titled "Vertical Agriculture" and created the term "vertical farming." Dickson Despommier created the idea of vertical farming in 1999 at Columbia University using new technologies. To ensure the secure growth of the chosen vegetables needed for a family, IIHR in India has designed a vertical garden structure that can be installed in the sun-bearing utility area, on the balcony, and on the terrace. The use of vertical farming also demonstrated how hydroponic vegetable crops could be produced on upper floors while poultry and fish that consume plant waste could be housed on lower floors. The problems facing vertical farming in India include public awareness, the agricultural community's inclusion, technical know-how, the expense of monitoring and maintaining the vertical farm systems, as well as its financial feasibility (Sonwane, 2018). Vertical

farming is the practise of growing plants, primarily vegetables, ornamentals, and herbs, without requiring soil, on a system of indoor shelves and utilising artificial light and fertiliser solutions. The practise of “vertical farming” involves growing food and medication in layers that are piled vertically, on surfaces that are inclined vertically, and integrated into other buildings like skyscrapers, old warehouses, and shipping containers. They also provide high-quality fresh fruit without relying on a favourable climate, fertile soil, or heavy water use, and they save labour, which is currently a precious resource. With nutritious supplements, vertical farming has the ability to feed the growing global population, particularly in urban settings.

World Scenario of vertical farming

Growing crops vertically in a controlled environment with technologies such as heating, lighting, air conditioning, ventilation, sensors and smart software, drones, the Internet of Things, and mobile applications is known as vertical farming. Urban farmers may easily grow vegetable crops by layering them vertically to conserve space and consume little irrigation energy and water (Shomefun et al., 2018). Growing lettuce plants in a hydroponic system using nutrient film technique (Sparks et al. 2018). The analysis of alternative energy scenarios revealed the possibility of energy consumption reductions of up to 53% and an increase in the overall system efficiency of vegetable production of up to a whopping 55% from the baseline. All across the world, many experiments are being conducted on vertical farming. It has already been introduced in the United State and Europe, Spain, Japan and Singapore. A number of technologically advanced vertical farms, including Aerofarms and Green Sense in the United States, Delicious in the Netherlands, Sharp’s strawberry farm in Dubai, Spread, Toshiba, and more than 100 additional vertical farms in Japan, as well as Packet Greens of Singapore, are successful examples of this type of farming. More than 50 farms are currently being run by INFARM throughout Berlin’s supermarket aisles, dining rooms, and distribution centres. Hydroponics has been identified by National

Aeronautics and Space Administration (NASA) experts as an appropriate technique for growing food in space. They have had success growing vegetables including radishes, lettuce, and onions.

Important features of vertical farming

- Vertical farms enable the producer to grow food 24 hours a day, 365 days a year.
- Obtained education and training to school children in food production by the vertical farming.
- Protect crops from unpredictable and harmful weather in vertical farming.
- In vertical farming, prevent crop loss from storage/shipping/ long transportation.
- In vertical farming, re-use of water collected from the indoor environment.
- Pride of producing food – an euphoric feelings.
- Vertical farming provide jobs for residents/communities.
- Stop agricultural runoff and water saving up to 90% in vertical farming.
- Minimize use of pesticides/fertilizers/ herbicides in vertical farming.

Advantages of Vertical Farming

1. Protection from Weather Problems

Vertical plant farming’s production can be largely independent of weather and shielded from severe weather events because it offers a stable climate.

2. Increased Crop Production

All year long, vegetables may be cultivated inside. All-season farming boosts the production of the farmed surface by a factor of 4 to 6 depending on the crop. Because there would be no need for transit between production and sale, there will be less chance of crop spoiling, pest infestation, and energy use than with traditional farming. Crops will be sold in the same facilities in which they were produced.

3. Growing Higher Quality Produce

Produce is of higher quality, has more nutritional value, and has a longer shelf life as there is no need to use toxic herbicides or pesticides.

Vertical Farming Flexibility

Over 80 different leafy greens and micro greens can be grown in vertical structure which may work on non-arable land and in close proximity to major markets or cities. From small to very large food operations, it's scalable.

Environment Friendly

Reduces the distance traveled by a considerable amount, reducing prices, energy consumption, and carbon footprint.

Conservation of Resources

Due to overpopulation, vertical farming would eliminate the need for new farmland, saving many natural resources. Agricultural encroachment on natural biomes will result in deforestation and desertification, which would be prevented.

Disadvantages of vertical farming

Initial high costly

The main issue is the initial high expense of establishing the vertical farming system. It will include the cost of building the buildings as well as the automation costs for things like computerised monitoring systems, remote control systems, automated racking and stacking systems, programmable LED lighting systems, climate control systems, etc.

Requirement of artificial light

Hugh energy cost as growing plant is entirely with artificial lights.

Contaminate water

The excess nutrients used in vertical farming may interfere and contaminate the main urban water system if not taken care of.

Create the problem maintain of temprature

LED lighting systems emit heat though small amount will create problem of maintaining the temperatures especially in summer months and may overload the air conditioning systems which will again incur high energy cost.

Dispose of residues

Lot of garbage, plant residues, etc. will be generated around the buildings with vertical farming which needs to be disposed off properly.

Required skilled labours

Skilled workforce will be unavailable initially and will need to be trained.

Systems or innovations of Vertical farming

- **Aeroponics:** In contrast to traditional hydroponics and aquaponics, aeroponics does not need liquid or solid plants to grow in plants. In aeroponics, nutrient-rich liquid is misted onto the plants rather than the air chambers where they are hung. In the 1990s, NASA promoted the use of aeroponics in their quest to develop effective methods for cultivating plants in space. With up to 90% less water usage than traditional hydroponic systems and no need for a replacement for the grown medium, aeroponics is by far the most environmentally friendly method for soil-free cultivation.
- **Aquaponics:** It is a bio-system that integrates hydroponic vegetable, flower, and herb production with recirculated fish aquaculture to foster symbiotic interactions between the plants and the fish. The nutrient-rich wastewater is filtered here by a solid removal unit and sent to a bio-filter, where hazardous ammonia is changed into nutritious nitrate. The hydroponic beds then serve the dual purpose of absorbing nutrients and purifying wastewater before it is recycled into the fish tanks. In order to conserve energy, the plants also absorb carbon dioxide from the fish, and the water heated in the fish tanks helps the greenhouse maintain its nighttime temperature.
- **Hydroponics:** Hydroponics is the practise of growing plants without soil in mineral and fertiliser solutions. In hydroponic systems, the roots of the plants are supported by inert materials like gravel, sand, or sawdust while being immersed in liquid solutions containing macronutrients. Hydroponics has the capacity to boost yield per area while utilising less water. One of hydroponics' main benefits is that it lessens soil-related cultivation issues including pests, diseases, and soil-borne insects.
- **Sky Greens:** The first hydraulically driven vertical farm in the world. The breakthrough

vertical farming system that Sky Greens, a Singapore-based business, has created is also the first low-carbon, hydraulically powered farm in the world. The veggies are grown on shelves that rotate continuously all day.

- **AeroFarms:** Another smart vertical farming innovation - the technology of growing greens without sun or soil under controlled environment.
- **Bowery:** The most technologically, sophisticated commercial indoor farm in the world. The technology automatically generates ideal conditions for the plants, while collecting the data as they grow. The data help in providing the plants with the exact amount of light, nutrients and water.
- **Cubic Farming Systems:** The next-generation sustainable farming system-working with conveyor rotation and automated nutrient delivery system.
- **VertiCrop:** A sustainable farming technique for urban areas.
- **Modular Farms:** Produce fresh produce virtually anywhere in the world.
- **Plantscapers:** A building that provides food for its occupants.
- **Zip Grow:** Vertical farming for the modern farmers involving conveyor rotation, automated nutrient delivery and LED lighting.

The development of vertical farming overseas has been beneficial for space farming, which would be in demand even on planets with either short-term or long-term habitat (Kheir Al-Kodmany, 2018).

Vertical Systems

Plants in vertical systems can be seen stacked vertically in rows while others are on the ground. Results from tests on crops with tiny plants, like lettuce, were inconsistent. A substrate-filled bag is penetrated by the nutrient solution, which is administered at the top. At the bottom is where the water that came from the drain is left. Using as much of the available area as possible to maximise yield per square metre is the major objective of this approach.

Vertical Towers

A tower garden, also referred to as a window farm, is a vertical hydroponics system made up of bottle cascades, an A-Frame hydroponic system, and a hydroponic wall. It may be used to cultivate a variety of plants, including kale, broccoli, spinach, Swiss chard, lettuce, and herbs. It may also be used to grow plants indoors if lights are placed above the tower, which is typical in cities with little space for gardening. You may change the design to suit your tastes. To collect the nutritional solution, for instance, towers with drains that may empty into a single tank can be suspended from the top (Dunn, 2017).

Lighting

A major problem with vertical farming is lighting. Whether the vertical farm is intended to use just artificial light entirely or mixed artificial and natural light, should be considered in order to manage the production line. The facility's design must take into account the same problems. There are two alternatives: HPS or LED (light emitting diode) (high-pressure sodium). The range of light intensity required to promote the development of vegetation depends on the location, time, temperature, and CO₂ concentration of the surrounding air. Taking into account all of these factors, the typical range of light intensity used is 50-200 mol/m²/s, or around 4100-16400 lx when high-pressure sodium lamps are included. In a confined environment, plant needs roughly 18 hours of light every day to flourish. They make use of light shelves to increase the amount of light that comes in. Each window can reach a maximum height of 3.5 metres, which corresponds to the height of each level.

Natural Lighting

The structure is designed to absorb as much light as possible. In areas with temperate weather, it is possible to build some elements, such as the roof, to take full use of the sun's energy throughout the year. There is a possibility that sunlight will also reach other areas of the structure. Any reduction in internal stacking density in a vertical farm is matched by an increase in building cubature. Although natural daylight is the

primary source of energy obtained from the outside, it is necessary to develop techniques for the concentration, direction, and distribution of daylight in order to utilize it efficiently throughout the year. The concept of the vertical farm may be used in several locations with various sorts of weather and lighting conditions. This idea's flexibility frequently results in modular building designs and the usage of a variety of architectural concepts. Additionally, it makes use of several façade components that include lighting-guiding concepts. Clearly, no one has ever recommended stacking solar panels on top of one another before. Similar to this, if there is not a suitable replacement for the necessary light, layers of plants cannot be built on top of one another.

Solar Cell

The system's functions must all be extremely effective, but notably plant photosynthesis. Non-PAR waves (Photosynthetically Active Radiation), which are not absorbed by chlorophyll, should be filtered in order to maximise the utilisation of solar energy in vertical farms. Selected solar cells must filter these waves before using them to generate photovoltaic energy. Recently, many semi-transparent cells have been created. However, lesser efforts have been made to make such cells particularly transparent for wavelengths that are active in photosynthetic processes. Desensitized solar cells may be created by using the red/purple hues with the maximum degree of absorption (535 nm) as its foundation. Because the vertical farming multiplies the area of the vegetation growth by the number of stories, the PAR derived from natural sources is insufficient. Solar energy must be collected from the structures all around the city by a system of mirrors in order to reduce the demand for additional artificial light. This would improve the performance of selected PV systems and support PAR in a vertical farm. What the energy model revealed was that some components of the site may provide enough energy to meet the facility's demands for both light and water within a month. The results demonstrated that sufficient energy may be obtained from solar panels installed on building facades or rooftops where they can capture sunlight and have access to pumping facilities. The third dimension was quite useful

and required illumination. Due to the loss of energy caused by the final three dimensionality, more panels than the structure could carry were required.

LED

As previously stated, in addition to natural light, artificial lighting is also necessary, and one such source may be LEDs (Light Emitting Diodes). Longer-lasting and less expensive LEDs are good alternatives. Although there are numerous options, LEDs are being utilised more frequently as a plant light source to aid with night interruption or Day Extension (DE). They have a long lifespan, are energy-efficient, and can target certain light wavelengths to better control the photoperiod. This may be accomplished by employing LED bulbs, which are made up of a number of dimmable diodes with different colour spectrums. This kind of lamp may be controlled to create light that is appropriate for plants. LED technology reduces energy consumption by modifying the spectrum and intensity of light using a process developed at Chalmers University. The ability of LEDs to control the ratio of red and far-red colours to elicit the greatest responses from plants is another benefit. The stem elongation is also enhanced if this fraction is low. Plants are shadowed by others around them in this shade avoidance approach. The best combination of red/blue and R/FR is currently being researched. However, further study is required on a variety of attractive plants. As an instance, just red light is used for lettuce. There was a poor correspondence between high-pressure sodium lamps and the spectrum used by plants during photosynthesis. Plants don't get nearly enough of the red and blue light they require. However, they also receive a lot of yellow light, which is not very useful, and a lot of infrared radiation, which is bad for some items. In a regulated setting for agriculture, companies like NASA looked into LEDs and discovered that they were the greatest lights for creating a favourable atmosphere for plants to flourish. Red light is necessary for photosynthesis, whereas blue light helps to provide the ideal conditions for plant morphogenesis. All of this is done to facilitate food production in restricted environments. The ideal colours for LEDs are red and blue.

Control Environment System

When designing a vertical farming system, considerations for temperature, air conditioning, and ventilation are crucial. The advantages of a heating, ventilation, and air conditioning system make it appropriate for vertical farming, including improved interior air quality, energy efficiency, moisture uniformity, and heat in vertical farms given by plant shading. The structure that consumes the least amount of energy is the most effective at conserving energy. cooling of the structure that is dependent on the vertical farm's geographic locations. The use of regenerated energy requires adequate and logical distribution and transfer systems. This process is recommended to include the possibility of using ground water or surface water, as well as the utilisation of geothermal technology, heat pumps, and solar energy plants for heating and cooling. Systems supported by water move heat loads. Earth canals are used in conjunction with air collectors to regulate the necessary healthy level of outdoor air in order to achieve regenerative energy. The internal heating conditions in each segment of a vertical farm are continuously monitored.

Smart Devices

As a totally automated operation, vertical farming makes a great use of sensors and actuators that also interact with other systems with no human interference. A complete calculating system that continually considers the environment and helps in the generation of appropriate information and services is required in order to implement vertical farming as a technology. A database exists that contains all relevant data on crops and potential illnesses. Even in situations when crops are grown indoors, external weather is required for the ventilation system. Real-time weather forecasts provide information about the weather, and appropriate judgments may be taken in light of this knowledge. To choose the controller appropriately, one has to have knowledge of the context. Water required

For modern farming, 70% of the available freshwater would be used. A significant portion of this loss is caused by the artificial irrigation of fields, and the majority of irrigation water is lost to

evaporation. Although evaporation is a normal process, there is a greater issue with the water that leaves the farm as runoff and is not fit for human use. Because of the above rationale, less water is wasted when farms are moved indoors, which may be used for plant growth instead. According to estimates, hydroponic farming requires one litre of water per square foot each day. To combat the scarcity of water, vertical farming employs a variety of strategies.

Renewable energy in vertical farming

The current energy needs to be measured in order to determine the quantity of energy needed to power a target building and whether or not reusable energy can meet the building's goal demand. The following are required for a vertical farm: lighting, temperature, ventilators, heat pumps to control the environment, pumps to mix nutrients, and agitators to manage plants. Renewable technology, which first appeared a long time ago as very transformative, oversaw vertical farming.

Popular vertical farming of vegetable in india

Cucurbits Trained Vertically

Viny cucurbits planted on the ground take up a lot of room but are not very productive. In vertical gardens, vines are trained to climb trellises or garden nets, allowing for a bigger plant population and more output than in open fields. The same is carried out in a soil or soilless media under safe circumstances. It works best with various beans, yard-long cow peas, and indeterminate tomato kinds. The cucumber, bottle gourd, sponge gourd, ridge gourd, snake gourd, and bitter gourd are essential cucurbit crops for vertical farming.

Micro Greens

A relatively new speciality that is quickly gaining popularity among urbanites who are health-conscious is microgreens and baby greens. Vegetables and herbs that are still in the early stages of development make up these crops. High levels of phytochemicals that promote health are also present in certain species of microgreens. Vertical farming may be used to grow microgreens inside, outside, during and outside of the growing

season, in homes, and on farms. They are favoured by cooks and diners to improve a dish's flavour, colour, and texture. Due to their quick growth (7 to 21 days), quick germination, powerful flavour, vibrant colour, and therapeutic benefits, microgreens of the genus Brassica, mainly broccoli, have recently gained popularity. Currently, commercial producers are adopting a vertical method in greenhouses and soilless medium in trays to produce microgreens. Similar to nutrient film technology systems, capillary mats laid in troughs are used to hydroponically grow microgreens as well.

Conclusion

Vertical farming is unquestionably a solution to major issues in Indian agriculture, including the lack of or plenty of agricultural products, excessive use of pesticides and fertilisers, degrading soils, and even unemployment. Challenges do exist, nevertheless, such as the Indian farming community's resistance to vertical farming. The absence of daytime electrical supply, the lack of minimum support prices, the inability to regulate the market surplus, the paucity of water, and other issues are just a few of the issues that Indian farmers must deal with. Implementing vertical farming in India has several challenges, not the least of which is the initial high cost of infrastructure for a large-scale farm. Other obstacles that vertical farming in India must overcome include public awareness, the agricultural community's inclusion, technical know-how, costs associated with operating and maintaining the vertical farm systems, and its feasibility from an economic standpoint.

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MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF EIGHT BREAD WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES TO TERMINAL HEAT STRESS UNDER TIMELY AND LATE SOWN CONDITIONS

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Abstract

Terminal heat stress driven by global warming is among the most serious constraints to wheat productivity, particularly in the Indo-Gangetic plains where late planting exposes the reproductive phase to high temperature. A field experiment was conducted during the *rabi* season of 2024–25 at Swami Vivekanand Subharti University, Meerut, to evaluate eight bread-wheat genotypes (K-65, K-816, K-8434, K-9351, PBW-154, PBW-343, PBW-550 and PBW-226) under timely sown (control; 25 November) and late sown (terminal heat stress; 15 December) conditions in a factorial randomized design with three replications. Yield and yield-contributing traits, leaf chlorophyll (SPAD), and the osmolytes proline and total soluble sugar were recorded and analysed by two-factor analysis of variance. Late sowing significantly ($p = 0.01$) reduced plant height, tillers plant⁻¹, spike length, spikelets spike⁻¹, grains spike⁻¹, 1000-grain weight, days to maturity and grain yield plant⁻¹, the largest mean reductions occurring for days to maturity (~11.0%) and grain yield (~10.2%). Conversely, proline and total soluble sugar increased markedly under heat stress (overall increases of ~53% and ~54%, respectively), reflecting active osmotic adjustment. Genotype, sowing date and their interaction were significant for almost all traits. PBW-550 was the most heat-tolerant genotype—recording the smallest reductions in yield, grain weight and chlorophyll together with the highest osmolyte accumulation—followed by K-9351 and K-816, whereas PBW-343 was the most susceptible. Grain yield under late sowing was positively associated with retained chlorophyll and osmolyte accumulation. PBW-550 is therefore recommended for late-sown, heat-prone environments.

Keywords: *Triticum aestivum*; terminal heat stress; late sowing; chlorophyll (SPAD); proline; total soluble sugar; grain yield; genotype × environment interaction.

Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops and a cornerstone of food and nutritional security, supplying a major share of dietary energy, protein and fibre. India is the second-largest producer globally, yet productivity gains are increasingly threatened by rising temperatures. Heat stress

associated with global warming already affects a large fraction of the world's wheat-growing area, and each 1 °C rise in seasonal temperature is associated with appreciable yield decline (Kumar *et al.*, 2023). In the Indo-Gangetic plains, delayed planting after rice harvest commonly pushes anthesis and grain filling into the warmer months, exposing the crop to terminal heat stress that

curtails the grain-filling duration and depresses yield (Sihag *et al.*, 2024).

High temperature during the reproductive phase impairs photosynthesis, accelerates leaf senescence, reduces pollen viability and grain set, and shortens grain filling, collectively reducing grain number, grain weight and yield (Alghabari *et al.*, 2021; Miroslavjević *et al.*, 2024). At the biochemical level, heat-tolerant genotypes typically accumulate higher concentrations of compatible osmolytes such as proline and soluble sugars, which contribute to osmotic adjustment, membrane protection and reactive-oxygen-species detoxification (Sihag *et al.*, 2024; Satbhai *et al.*, 2025). The retention of leaf chlorophyll—the stay-green trait—is widely used as an indicator of heat tolerance because it sustains source activity and photosynthetic capacity under stress (Anwar *et al.*, 2024).

Because the heat-stress response is governed by many traits and is strongly influenced by genotype-by-environment interaction, screening diverse germplasm under contrasting sowing dates remains an effective route to identify tolerant cultivars and the most suitable sowing window for each genotype (Alsamadany *et al.*, 2023; Sihag *et al.*, 2021). The present study was therefore undertaken to characterise the morpho-physiological and biochemical responses of eight bread-wheat genotypes under timely and late sown conditions during 2024–25, and to identify genotypes combining stable yield with strong physiological and osmolyte-based tolerance to terminal heat.

Materials and Methods

Experimental site, design and treatments

The field experiment was conducted during the *rabi* season of 2024–25 at the experimental farm of the Department of Biotechnology, Keral Verma Subharti College of Science, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India. Eight bread-wheat genotypes—K-65, K-816, K-8434, K-9351, PBW-154, PBW-343, PBW-550 and PBW-226—were evaluated under two sowing environments: a timely sown control (TS; 25 November) and a late sown terminal-heat treatment (LS; 15 December), the latter imposed

to expose the reproductive and grain-filling phases to elevated temperature. The experiment was laid out in a two-factor (genotype × sowing date) randomized design with three replications. The recommended package of agronomic practices, fertilisation and need-based irrigation was followed uniformly across plots.

Morphological and yield observations

At physiological maturity, plant height, number of tillers plant⁻¹, spike length, number of spikelets spike⁻¹, number of grains spike⁻¹, 1000-grain (test) weight, days to maturity and grain yield plant⁻¹ were recorded from randomly selected competitive plants following standard procedures.

Chlorophyll and biochemical estimation

Relative leaf chlorophyll content of the flag leaf was measured non-destructively with a SPAD chlorophyll meter at 50, 70 and 90 days after sowing (DAS) under both environments. Free proline was estimated by the ninhydrin method of Bates *et al.* using sulphosalicylic-acid extraction and absorbance at 520 nm, and total soluble sugar was determined by the anthrone method with absorbance at 620 nm, both expressed on a fresh- and dry-weight basis, respectively, as appropriate.

Statistical analysis

All traits were subjected to a two-factor analysis of variance (ANOVA) appropriate to the genotype × sowing-date structure. Treatment, genotype and interaction effects were tested with the F-statistic. The standard error of the mean (SEm ±), the critical difference (CD = SEd × t_{0.05}) and the coefficient of variation (CV %) were computed for each trait. Effects were considered significant at p = 0.05; * and ** denote significance at p = 0.05 and p = 0.01, respectively, and NS denotes non-significance.

Results and Discussion

Yield and yield-contributing traits

Analysis of variance revealed highly significant (p = 0.01) effects of sowing date and of genotype for all eight agronomic traits, and a significant genotype × sowing-date interaction for most of them (Table 1). Late sowing reduced every trait relative to the timely sown control. Mean plant

height declined from 96.2 to 86.1 cm, spike length from 10.13 to 9.05 cm, grains spike¹ from 48.2 to 42.7, 1000-grain weight from 41.54 to 37.42 g, and grain yield plant¹ from 16.81 to 15.10 g. The shortening of the life cycle was pronounced, with mean days to maturity falling from 130.3 to 116.0 days, consistent with heat-accelerated senescence and a compressed grain-filling period (Sihag *et al.*, 2024; Mirosavljević *et al.*, 2024).

Among genotypes, PBW-550 consistently showed the smallest proportional reductions under late sowing for grain yield, 1000-grain weight, spike length and days to maturity, followed by K-9351 and K-816, whereas PBW-343 sustained the largest reductions and was the most heat-susceptible. This ranking agrees with reports that tolerant cultivars better preserve spike fertility and grain weight under terminal heat through stay-green and efficient assimilate remobilisation (Alghabari *et al.*, 2021; Anwar *et al.*, 2024). The low coefficients of variation (0.90–4.83%) indicate good experimental precision.

Table 1. Effect of sowing date on yield and yield-contributing traits of eight wheat genotypes during 2024–25. TS = timely sown (control); LS = late sown (terminal heat stress). ** = significant at $p = 0.01$; * = $p = 0.05$; NS = non-significant. F (sowing), F (genotype) and F (G×S) are the variance ratios for sowing date, genotype and their interaction.

Genotype	Plant height (cm)		Tillers plant ¹		Spike length (cm)		Spikelets spike ¹	
	TS	LS	TS	LS	TS	LS	TS	LS
K-65	115.9	104.5	7.83	6.60	10.16	9.16	20.2	17.6
K-816	80.1	72.5	8.73	7.84	9.46	8.73	21.8	19.6
K-8434	107.2	93.4	7.04	6.07	10.84	9.52	21.9	19.7
K-9351	103.1	95.9	8.87	8.38	10.60	9.62	21.0	19.3
PBW-154	90.1	81.2	7.92	7.12	9.82	8.95	19.8	17.5
PBW-343	95.6	78.9	6.81	5.40	9.13	7.29	19.1	15.4
PBW-550	82.0	78.9	9.14	8.77	11.23	10.69	21.9	20.5
PBW-226	95.3	83.3	7.53	6.30	9.83	8.47	20.2	17.2
Mean	96.2	86.1	7.99	7.06	10.13	9.05	20.7	18.3
SEm (±)	0.333		0.069		0.059		0.121	
CD (p=0.05)	0.959		0.198		0.169		0.348	
CV (%)	1.79		4.47		3.00		3.03	
F (sowing)	456.64**		91.09**		168.98**		197.62**	
F (genotype)	290.08**		55.17**		50.87**		32.01**	
F (G×S)	10.29**		1.78 NS		3.06*		2.33**	

Genotype	Grains spike ¹		1000-grain weight (g)		Days to maturity		Grain yield plant ¹ (g)	
	TS	LS	TS	LS	TS	LS	TS	LS
K-65	48.7	43.6	45.22	39.54	130.7	116.2	18.46	16.19
K-816	44.5	42.0	39.32	36.40	126.1	115.1	15.13	14.27
K-8434	53.3	45.3	43.95	38.11	133.8	115.8	17.51	15.28
K-9351	50.3	45.8	43.21	40.59	129.0	120.0	18.14	17.40
PBW-154	44.7	41.0	39.25	36.15	126.1	114.5	16.42	14.66
PBW-343	42.0	34.5	38.83	31.74	136.7	111.4	14.89	11.96
PBW-550	54.4	50.1	41.31	40.27	132.6	126.2	17.08	16.44
PBW-226	47.8	39.6	41.27	36.59	127.7	109.0	16.87	14.60
Mean	48.2	42.7	41.54	37.42	130.3	116.0	16.81	15.10
SEm (±)	0.200		0.155		0.227		0.102	
CD (p=0.05)	0.575		0.447		0.653		0.293	
CV (%)	2.15		1.93		0.90		3.12	
F (sowing)	374.88**		352.92**		1996.06**		141.74**	
F (genotype)	120.49**		62.41**		56.78**		49.10**	
F (G×S)	7.26**		10.67**		45.61**		4.51**	

Biochemical responses: proline and total soluble sugar

In contrast to the agronomic traits, the osmolytes proline and total soluble sugar increased significantly under late sowing (Table 2). Mean proline rose from 0.259 to 0.397 $\mu\text{g g}^{-1}$ fresh weight (an increase of about 53%) and mean total soluble sugar from 4.22 to 6.50 mg g^{-1} dry weight (about 54%), with highly significant sowing-date, genotype and interaction effects. The heat-tolerant genotype PBW-550 recorded the highest osmolyte accumulation under stress (proline 0.507 $\mu\text{g g}^{-1}$; soluble sugar 8.05 mg g^{-1}), followed by K-9351 and K-816, whereas the susceptible PBW-343 accumulated the least. Greater accumulation of compatible solutes in tolerant genotypes supports osmotic adjustment, protects membranes and scavenges reactive oxygen species, and is a well-documented hallmark of terminal-heat tolerance in wheat (Sihag *et al.*, 2024; Alsamadany *et al.*, 2023; Satbhai *et al.*, 2025).

Table 2. Effect of sowing date on proline and total soluble sugar content of eight wheat genotypes during 2024–25. TS = timely sown; LS = late sown (heat stress). ** = $p = 0.01$.

Genotype	Proline ($\mu\text{g g}^{-1}$ FW)		Total soluble sugar (mg g^{-1} DW)	
	TS	LS	TS	LS
K-65	0.264	0.411	4.20	6.48
K-816	0.268	0.434	4.64	7.29
K-8434	0.251	0.354	3.79	5.70
K-9351	0.259	0.421	4.63	7.50
PBW-154	0.259	0.391	3.98	6.40
PBW-343	0.235	0.311	3.73	4.96
PBW-550	0.289	0.507	4.89	8.05
PBW-226	0.247	0.350	3.94	5.58
Mean	0.259	0.397	4.22	6.50
SEm ($\pm$)	0.003		0.053	
CD ($p=0.05$)	0.008		0.152	
CV (%)	4.32		4.83	
F (sowing)	1143.26**		922.54**	
F (genotype)	43.41**		49.57**	
F (G×S)	15.20**		9.37**	

Leaf chlorophyll content (SPAD)

Leaf chlorophyll (SPAD) declined with crop age in both environments and was further reduced by terminal heat, the reduction widening from 50 to 90 DAS as senescence advanced (Table 3). At 90 DAS the mean SPAD value fell from 46.3 under timely sowing to 39.9 under late sowing. The smallest stress-induced reduction in chlorophyll was recorded in PBW-550 and K-9351, which retained green leaf area longest (stay-green), while PBW-343 showed the steepest decline. Maintenance of chlorophyll under heat sustains photosynthetic source activity during grain filling and was positively associated with grain yield across genotypes, in agreement with previous terminal-heat studies (Anwar *et al.*, 2024; Kumar *et al.*, 2023).

Table 3. Leaf chlorophyll content (SPAD units) of eight wheat genotypes at 50, 70 and 90 days after sowing (DAS) under timely and late sown conditions during 2024–25.

Genotype	Timely sown (control)			Late sown (heat stress)		
	50 DAS	70 DAS	90 DAS	50 DAS	70 DAS	90 DAS
K-65	50.3	49.1	44.9	47.9	44.6	37.9
K-816	51.6	48.9	47.1	49.2	46.9	42.3
K-8434	51.2	49.8	46.4	50.0	44.7	39.4
K-9351	53.6	51.9	48.2	51.6	48.6	44.0
PBW-154	49.9	50.7	45.7	48.6	45.2	40.4
PBW-343	49.1	47.4	45.0	45.8	40.9	35.2
PBW-550	52.9	51.4	47.0	50.9	49.0	44.0
PBW-226	51.6	50.5	47.0	48.3	44.2	38.1
Mean	51.3	50.0	46.4	49.0	45.5	40.2
SEm ($\pm$)	0.31	0.36	0.40	0.31	0.36	0.40
CD ($p=0.05$)	0.90	1.05	1.16	0.90	1.05	1.16
CV (%)	1.49	1.81	2.20	1.49	1.81	2.20

Conclusion

Terminal heat stress imposed through late sowing significantly impaired the growth, yield components and grain yield of all eight wheat genotypes during 2024–25, while triggering compensatory accumulation of proline and total soluble sugar and accelerating chlorophyll loss and maturity. The largest mean penalties were observed for days to maturity and grain yield. Genotypic variation and genotype $\times$ sowing-date interaction were significant for almost all traits, confirming that heat tolerance is cultivar-specific. PBW-550 emerged as the most heat-tolerant genotype—combining the smallest reductions in yield, grain weight and chlorophyll with the highest osmolyte accumulation—followed by K-9351 and K-816, whereas PBW-343 was the most susceptible. PBW-550 can therefore be recommended for cultivation under late-sown, heat-prone conditions, and the osmolyte and stay-green traits identified here may serve as useful physiological selection criteria in heat-tolerance breeding (Sihag *et al.*, 2021; Tanin *et al.*, 2022).

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IMPACT OF INTEGRATED NUTRIENT MANAGEMENT ON YIELD PARAMETERS OF ONION (*ALLIUM CEPA* L.) CV. PUSA RED

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Abstract

The present study was conducted to find out the effect of integrated nutrient management (INM) on yield parameters of onion (*Allium cepa* L.) Cv. Pusa Red under Western Uttar Pradesh condition in the year 2019-20 and 2020-21 during *rabi* season at Horticultural Research Farm, Department of Horticulture, Chaudhary Charan Singh University, Meerut (U.P) India. The experiment was laid out in randomized block design with three replications and a total number of fourteen treatments were evaluate for yield and its attributing parameters of onion. Among all the treatments, soil application of 75% RDF through chemical fertilizers with Vermicompost @ 12.5 q/ha & Azospirillum @ 5 kg/ha was found significantly superior over all other treatments concerning yield. Maximum number of scales per bulb (7.17), diameter of bulb (6.86 cm), fresh weight of bulb (46.39 g), dry weight of bulb (21.47 g) and yield (308.54 q/ha) were recorded with treatment T₈ (75% RDF + Vermicompost @ 12.5 q/ha + Azospirillum @ 5 kg/ha). Whereas, minimum number of scales per bulb (4.04), bulb diameter (4.66 cm), fresh weight of bulb (36.03 g), dry weight of bulb (9.05 g) and yield (239.63 q/ha) was observed with the treatment T₁ (control).

Key words: Onion, RDF, Vermicompost, Azospirillum, Yield

Introduction

Onion (*Allium cepa* L.) is one of the oldest bulb crops known to mankind and it is consumed worldwide. It is a member of the Alliaceae family, with the Near East and Mediterranean serving as secondary centers of origin. It was brought to India from Palestine (Yadav et al., 2013). Onion commonly known as “Queen of the kitchen” (Selvaraj, 1976; Griffiths et al., 2002) contains vitamin B, vitamin C and also traces of iron and calcium. As a culinary ingredient, it enhances the flavor and taste of a variety of food preparations. It is also used in salads and contains steroidal saponins (Carotenuto et al., 1999), which stop the intestines from absorbing cholesterol (Ahmed et al., 2020). It also contains vital nutrients and phytochemicals that are good for health. It has been demonstrated that regular onion consumption lowers the risk of heart disease, vascular disease, cancer, cataracts, and DNA

damage (Arung et al., 2011; Jimenez et al., 2011; Hamauzu et al., 2011).

Onion is an essential commodity of commerce, owing to a special pungency it is more valuable than any other vegetable (Dutt, 2017). The pungency in onion is due to presence of volatile compound known as allyl propyl disulphide (C₆ H₁₂ S₂). The red colour of onion bulbs is due to the presence of the pigment anthocyanin. Onion has great medicinal significance referred by Bhandari (1986) in his book encyclopedia of Indian herbs under the chapter ‘Vanaushadhi Chandrodya’.

Integrated nutrient management (INM) offers an effective strategy (Dimri and Singh, 2005) by the combined application of organic manures and inorganic fertilizers to increase yield of onion crop. INM practice is important to supply all essential nutrient elements required by the plants for optimum growth and development. Chemical fertilizers which are artificially synthesized

having high concentration of nutrient elements, these chemical fertilizer are rapidly release in soil and easily uptake by the plant roots. The uptakes of nutrients by plants involve several biochemical and physiological processes occurring both within and outside the plant roots (Mengel and Kirkby, 1980).

Organic manures like farmyard manure and vermi-compost are available in plenty in locality and can be efficiently utilized for vegetable production. Besides this, bio-fertilizers are renewable source of plant nutrients to supplement the chemical fertilizers. Farmyard manure is explained as a perfect source of nutrients for plant growth as well as for soil micro-biota. It is one of the efficient and effective organic manure. It can provide organic matter to soil microbes as a source of carbon. An increase in microbial population leads to the degradation of pesticides and heavy metals to less harmful compound. Application of farmyard manure not only increase the availability of nutrients in the soil but also improves the soil properties like soil structure, water holding capacity, bulk density, cation exchange capacity, etc. (Chahal *et al.*, 2020).

Bio-fertilizers are important component of integrated nutrient management, as they are renewable source of plant nutrient elements. Biofertilizers are beneficial living micro-organism which have great significant to enhance crop growth and production through biological nitrogen fixation. Kurrey *et al.* (2018) reported that azotobacter enhance onion yield through increase the availability of nitrogen. Azospirillum is an another nitrogen fixing bacterium that promotes plant growth as identified in most studied on different crop plants (Raffi *et al.*, 2021).

Materials and Methods

The experiment was conducted to study the impact of integrated nutrient management on yield parameters of onion (*Allium cepa* L.) cv. Pusa Red during *Rabi* season at Horticultural Research Farm, Department of Horticulture, Chaudhary Charan Singh University Campus, Meerut (U.P.) India, during the years 2019-20 and 2020-21. The treatments involved in the study

were fourteen in numbers i.e. T₁ (control), T₂ (Recommended dose of NPK/ha (100:80:50 kg), T₃ (75% RDF + FYM @ 5 t/ha), T₄ (75% RDF + FYM @ 5 t/ha + Azotobacter @ 5 kg/ha), T₅ (75% RDF + FYM @ 5 t/ha + Azospirillum @ 5 kg/ha), T₆ (75% RDF + Vermicompost @ 12.5 q/ha), T₇ (75% RDF + Vermicompost @ 12.5 q/ha + Azotobacter @ 5 kg/ha), T₈ (75% RDF + Vermicompost @ 12.5 q/ha + Azospirillum @ 5 kg/ha), T₉ (50% RDF + FYM @ 10 t/ha), T₁₀ (50% RDF + FYM @ 10 t/ha + Azotobacter @ 5 kg/ha), T₁₁ (50% RDF + FYM @ 10 t/ha + Azospirillum @ 5 kg/ha), T₁₂ (50% RDF + Vermicompost @ 25 q/ha), T₁₃ (50% RDF + Vermicompost @ 25 q/ha + Azotobacter @ 5 kg/ha) and T₁₄ (50% RDF + Vermicompost @ 25 q/ha + Azospirillum @ 5 kg/ha). The experiment was laid out in randomized block design (RBD) and replicated three times. The experimental area was under subtropical climatic condition having summer with maximum temperature 36.5°C, minimum temperature 7.30°C in winter and relative humidity ranging from 30-90 per cent in different seasons of the year. The soil of experimental field had pH of 7.35. Seeds of onion cv. Pusa Red were sown on nursery beds prepared earlier. The soil of seed bed was prepared with compost and mulching was protecting the young seedlings from adverse climatic condition. Covering materials were removed from the bed after seed germination (7-10 days after sowing) for optimum growth of seedlings. 40 DAS seedlings were ready for transplanting. The healthy seedlings having uniform growth were selected and transplanted on well prepared field at afternoon. The field was prepared by ploughing with disc plough and subsequent ploughing was done with cultivator followed by levelling. The required area was then marked and plots were prepared according to the layout plan. The soil of the experimental site was irrigated before transplanting for optimum moisture. Seedlings were treated with Thiram @ 2g/kg. After proper treatment the selected seedlings were transplanted in field at a spacing of 10 x 15 cm. From every plot 10 plants were taken randomly for recording of observations. The observations were recorded for number of scales per bulb, diameter of bulb, Fresh weight of bulb, dry weight of bulb and yield.

Result and Discussion

Nutrients supplied through chemical fertilizers, organic manures and biofertilizers are capable of redistribution of dry matter in the plant thereby bringing about an important in yield that depends not only on the accumulation of photosynthesis during crop growth and development but also on its partitioning in the desired storage organs. In the present study, yield attributes like, number of scales per bulb, diameter of bulb, fresh weight of bulb, dry weight of bulb and total yield were significantly influenced by integrated nutrient management.

Based on pooled data, the maximum number of scales per bulb (7.17) was recorded under the treatment T₈ (75% RDF + Vermicompost @ 12.5 q/ha + Azospirillum @ 5 kg/ha), while the minimum number of scales (4.04) was recorded with control after harvesting. This might be due to the soil application of chemical fertilizers, vermicompost and azospirillum which supply a good amount of NPK and micronutrients and also improve the physical & chemical properties of the soil. Since a sufficient supply of nutrient is thought to be essential for fostering the quick vegetative growth of plants, doing so will increase the bulb size of onion. In addition of these essential and better soil conditions further aid in the transfer of photosyntheter in the bulb storage organ, increasing bulb's size and number of scales per bulb. Early stimulation of the spares root system through effectual translocation of specific growth promoting compounds formed as a result of the protoplasmic activity of the tops in 75% RDF + Vermicompost 12.5 q/ha + Azospirillum 5 kg/ha treated onion plant. Adequate nutrient supply causes a denser rooting system, which results in an improvement in yield attributing characteristics and yield (Sharma *et al.*, 1994).

An examination of the data revealed that the integrated nutrient management study significantly influenced the bulb's diameter during both years of investigation. The maximum diameter of bulb (6.86 cm) was recorded under the treatment T₈ (75% RDF + Vermicompost @ 12.5 q/ha + Azospirillum @ 5 kg/ha), while the minimum diameter of bulb (4.66 cm) was recorded with control after harvesting based on pooled data. The larger diameter of onion bulb might be due to

the fact the organic manures such as vermicompost & FYM would have provided the macro nutrients and micronutrients and improved the physical and biological properties of the soil. Vermicompost and FYM release the nutrients in slowly and steadily manner into the soil system enabling the plant to absorb the nutrients. The soil enriched with vermicompost provides additional substances that are not found in chemical fertilizers (Kale 1998) helps for increasing bulb diameter. Additionally, azospirillum and azotobacter increase the nitrogen status of soil through atmospheric nitrogen fixation process, which may have resulted in luxuriant bulb size when biofertilizers and inorganic fertilizers were applied together. The related findings were also reported by Bhandari, *et al.* (2012), Mahanthesh *et al.* (2005), Kanaujia *et al.* (2009), Vachan and Tripathi (2018).

Integrated application of organic and inorganic fertilizers significantly influenced the fresh weight of onion bulbs. The average fresh weight of bulb (pooled data) after harvesting was also found to be maximum with 75% RDF + Vermicompost @ 12.5 q/ha + Azospirillum @ 5 kg/ha (46.39 g), while the minimum average of bulb weight under control treatment was least (36.03 g). These results are in line with the findings of Maneesh Kumar (2015) and Vikram Singh *et al.* (2017). The combined use of inorganic, organic and biofertilizer may have increased nutrient uptake and led to a buildup of enough photosynthates, which allowed bulbs to grow larger (in diameter and weight) and ultimately resulted in an increase in average bulb weight. Nitrogen is one of the most consumed elements, it plays an essential function in assimilating and transferring to growing organs (Fathi&Zeidali,2021) and also effect, the processes of photosynthesis and materialization (Arvin, 2019).

The observations recorded on the dry weight of bulbs indicate that the application of integrated nutrient management had a significant effect over control at harvest. As regards the INM application, it was observed that the maximum dry weight of bulbs with the treatment of 75% RDF + Vermicompost @ 12.5 q/ha + Azospirillum @ 5 kg/ha (21.47 g), followed by 50% RDF + Vermicompost @ 25 q/ha + Azospirillum @ 5 kg/ha.

The minimum dry weight of bulbs was recorded under the control (9.05 g). The increasing dry weight of bulbs with the application of RDF, Vermicompost and Azospirillum is in agreement with the findings of Devi and Limi-Ado (2005), Singh and Singh (2018) in onion and Sevak *et al.* (2012) in garlic. The desirable effect of application of vermicompost and azospirillum with 75% RDF (through chemical fertilizers) promoting the dry weight of bulbs may be due to the fact that some micro nutrients and macro-nutrients increase the rate of photosynthesis and frequency of chloroplasts and improve the amino acid content of the onion bulb and thus increase the dry weight of onion bulbs.

The critical analysis of the data presented in Table indicates that the yield of bulbs was significantly influenced by the integrated nutrient management study during both years of investigation. The highest yield was recorded in 75% RDF + Vermicompost @ 12.5 q/ha + Azospirillum @ 5 kg/h (308.54 q/ha), which was found superior over 50% RDF + Vermicompost @ 25 q/ha + Azospirillum @ 5 kg/ha (303.34 q/ha) and 50% RDF + Vermicompost @ 25 q/ha + Azotobacter @ 5 kg/ha (298.88 q/ha) and all other integrated nutrient treatments for both the years. The minimum yield recorded (239.63 q/ha) in control treatment. The present findings are in agreement with the observations made by Shinde *et al.* (2013), Vani *et al.* (2018), Kumar *et al.* (2017), Khandelwal (2010), Reddy *et al.* (2011), Kumar *et al.* (2015), Yadav *et al.* (2015) and Gowda *et al.* (2007) in garlic. Similarly, Meena *et al.* (2016) while studying the effect of INM had also found maximum increase in bulb yield of onion cv. N-53 when NPK was applied with organic manure and biofertilizer. The findings of Vani *et al.* 2018 also confirmed the result of this study and observed maximum yield due to the application of recommended dose of

nitrogen through chemical fertilizers and vermicompost.

Total bulb production may have increased as a result of improved nutrient uptake, which sped up synthesis and affected the movement of photosynthates from source (leaves) to sink (bulb) (Singh *et al.*, 1997) The yield may have improved greatly as a result of micro nutrients fertilization through INM enhanced net photosynthesis on the one hand and greater photosynthesis mobilization towards depository stature on the other. Nitrogen aids in the transfer of photosynthates in the bulb's storage organ, increasing the bulb's width and weight. Moreover, the significant effect on bulb yield, as consequence of inorganic fertilizer vermicompost and azospirillum was attributed to the increased nutritional status of soil resulting in increased total yield of onion. As vermicompost and FYM improve organic carbon status of soil so ultimately helps in better colonization of beneficial micro-organism such as azospirillum and azotobacter. Use of biofertilizers like Azospirillum and Azotobacter not only fixed the atmospheric nitrogen but also release of some plant hormones such as auxin, gibberellins and cytokinins which helps to enhance yield of onion.

Conclusion

According to the study's findings, boosting yield during the rabi season can be achieved through comprehensive nutrition management. Applying 75% RDF + Vermicompost @ 12.5 q/ha + Azospirillum @ 5 kg/ha improved all yield components. The findings clearly demonstrated that in order to meet the nutrient requirements of onions throughout the rabi season, an integrated use of organic manures and biofertilizers in addition to synthetic fertilizers is necessary.

Table 1:- Effect of Integrated nutrient management on number of scales per bulbs and diameter of bulb of onion (*Allium cepa* L.) cv. Pusa Red.

Not.	Treatment	Number of scales per bulb		Pool data	Diameter of bulb (cm)		Pool data
		2019-20	2020-21		2019-20	2020-21	
T ₁	Control	4.04	4.05	4.04	4.86	4.46	4.66
T ₂	Recommended dose of NPK/ha (100:80:50 kg)	5.34	6.83	6.08	5.60	5.23	5.41
T ₃	75% RDF + FYM @ 5 t/ha	5.50	6.85	6.17	5.73	5.33	5.53
T ₄	75% RDF + FYM @ 5 t/ha + Azotobacter @ 5 kg/ha	5.75	7.23	6.49	6.03	6.63	6.33
T ₅	75% RDF + FYM @ 5 t/ha + Azospirillum @ 5 kg/ha	5.80	7.42	6.61	6.06	6.86	6.46
T ₆	75% RDF + Vermicompost @ 12.5 q/ha	5.64	7.09	6.36	5.86	6.03	5.94
T ₇	75% RDF + Vermicompost @ 12.5 q/ha + Azotobacter @ 5 kg/ha	5.78	7.42	6.6	6.06	6.96	6.51
T ₈	75% RDF + Vermicompost @ 12.5 q/ha + Azospirillum @ 5 kg/ha	6.17	8.17	7.17	6.40	7.33	6.86
T ₉	50% RDF + FYM @ 10 t/ha	5.53	6.87	6.2	5.80	5.90	5.85
T ₁₀	50% RDF + FYM @ 10 t/ha + Azotobacter @ 5 kg/ha	5.67	7.10	6.38	6.00	6.16	6.08
T ₁₁	50% RDF + FYM @ 10 t/ha + Azospirillum @ 5 kg/ha	5.74	7.11	6.42	6.03	6.53	6.28
T ₁₂	50% RDF + Vermicompost @ 25 q/ha	5.64	7.00	6.32	5.83	5.93	5.88
T ₁₃	50% RDF + Vermicompost @ 25 q/ha + Azotobacter @ 5 kg/ha	5.96	7.49	6.72	6.30	7.00	6.65
T ₁₄	50% RDF + Vermicompost @ 25 q/ha + Azospirillum @ 5 kg/ha	6.17	7.98	7.07	6.40	7.23	6.81
	CD at 5%	0.716	0.955		0.774	1.378	
	SE +	0.245	0.327		0.265	0.471	

Table 2:- Effect of Integrated nutrient management on fresh weight of bulb, dry weight of bulb and yield of onion (*Allium cepa* L.) cv. Pusa Red.

Not.	Treatment	Fresh weight of bulb (g)		Pool data	Dry weight of bulb (g)		Pool data	Yield (q/ha)		Pool data
		2019-20	2020-21		2019-20	2020-21		2019-20	2020-21	
T ₁	Control	37.29	34.77	36.03	8.75	9.35	9.05	248.02	231.24	239.63
T ₂	Recommended dose of NPK/ha (100:80:50 kg)	39.38	40.41	39.89	9.45	12.42	10.93	262.87	268.74	265.81
T ₃	75% RDF + FYM @ 5 t/ha	40.25	41.26	40.75	12.86	13.51	13.18	267.68	274.42	271.05
T ₄	75% RDF + FYM @ 5 t/ha + Azotobacter @ 5 kg/ha	42.63	43.33	42.98	18.95	18.20	18.57	283.48	288.14	285.81
T ₅	75% RDF + FYM @ 5 t/ha + Azospirillum @ 5 kg/ha	43.14	44.38	43.76	18.08	18.94	18.51	286.87	295.16	291.02
T ₆	75% RDF + Vermicompost @ 12.5 q/ha	41.63	42.75	42.18	16.71	17.23	16.97	276.85	284.32	280.59
T ₇	75% RDF + Vermicompost @ 12.5 q/ha + Azotobacter @ 5 kg/ha	43.66	44.46	44.06	19.90	20.08	19.99	290.35	295.69	293.02
T ₈	75% RDF + Vermicompost @ 12.5 q/ha + Azospirillum @ 5 kg/ha	46.02	46.77	46.39	21.70	21.25	21.47	306.03	311.04	308.54
T ₉	50% RDF + FYM @ 10 t/ha	40.51	41.73	41.12	15.82	15.29	15.55	269.43	277.52	273.48

T ₁₀	50% RDF + FYM @ 10 t/ha + Azotobacter @ 5 kg/ha	41.75	42.82	42.28	16.83	18.04	17.43	277.63	284.74	281.19
T ₁₁	50% RDF + FYM @ 10 t/ha + Azospirillum @ 5 kg/ha	42.00	43.00	42.5	17.45	18.09	17.77	279.30	285.95	282.63
T ₁₂	50% RDF + Vermicompost @ 25 q/ha	40.56	42.18	41.37	16.22	16.76	16.49	269.76	280.11	274.94
T ₁₃	50% RDF + Vermicompost @ 25 q/ha + Azotobacter @ 5 kg/ha	44.29	45.59	44.94	21.34	20.74	21.04	294.57	303.19	298.88
T ₁₄	50% RDF + Vermicompost @ 25 q/ha + Azospirillum @ 5 kg/ha	45.30	46.02	45.76	21.59	20.76	21.17	301.72	304.95	303.34
	CD at 5%	0.644	0.635		2.115	1.852		4.286	5.475	
	SE +	0.220	0.217		0.724	0.634		1.466	1.873	

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PREVALENCE AND PHENOTYPIC CHARACTERIZATION OF CARBAPENEM-RESISTANT *ESCHERICHIA COLI* IN CLINICAL SPECIMENS FROM A TERTIARY CARE HOSPITAL IN NORTH INDIA

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Abstract

Carbapenem-resistant *Escherichia coli* is now a recognised global threat to human health due to the emergence and increasing prevalence of this resistance. To assess the original prevalence and phenotypic characterisation of carbapenem-resistant *E. coli*, the aim of this study was to detect carbapenemase production in *Escherichia coli* by the Disk diffusion (Kirby-Bauer method). The present study was carried out in the Department of Microbiology, Keral Verma Subharti College of Science, Meerut (U.P) and clinical samples were collected from the patients attending Subharti Medical College & Hospital, Meerut (U.P). A total of 206 *E.coli* isolates were examined regarding age, sex, departments and Carbapenem resistance among different clinical specimens such as Urine, Blood, and Pus received in the microbiology laboratory. It was observed that out of 206 *E.coli* isolates, about 58 (28.15%) isolates were from male patients, while 148 (71.84%) were from female patients. The majority (70.87%) of isolates were from urine samples. Out of 206 *E. coli* isolates, a total of 64 (31.06%) were carbapenem-resistant. The prevalence of *E. coli* that is resistant to carbapenem is rapidly increasing and is now the most significant issue in the field of infectious illnesses. Therefore, it is now crucial to identify the shifting pattern of resistance as soon as possible to prevent the spread of resistant germs and modify treatment plans.

Keywords: *Escherichia coli* (*E.coli*), Carbapenem Resistant *E. coli* (CREC), Antibiotic susceptibility testing, Multi-drug Resistance (MDR), Phenotypic method

Introduction

Carbapenem-type antibiotics are some of the newest and most effective beta-lactam antibiotics for fighting off bacteria. They have the broadest range of antimicrobial activity. Since the early 2000s, we have seen an increased incidence of community-acquired *E. coli* bacteria producing extended-spectrum beta-lactamases (ESBLs),

which are enzymes that will kill almost all types of beta-lactam antibiotics, except for carbapenems²³. This has resulted in an increased use of the antibiotic class, carbapenems. The use of carbapenems is critically important in treating patients with serious hospital-acquired infections, many of whom are the result of organ transplants, intensive care unit (ICU) admissions, and major surgical procedures. In addition, as carbapenems

have become more widely used and available, there is a growing threat to public health due to the emergence and spread of carbapenem-resistant Enterobacteriaceae (CRE). The ongoing threat of CRE threatens all that we have accomplished in health care in modern times. Compounding this public health concern is the minimal amount of new antibiotics being developed. As such, the global threat of antibiotic resistance to carbapenems is critical and requires urgent attention⁴.

Escherichia coli is an enteric bacterium, gram-negative and can exist as a facultative anaerobe. The bacteria belong to the family Enterobacteriaceae, which contains many species of bacteria. *Escherichia coli* bacteria are part of the normal flora of the intestines of all warm-blooded animals, including humans²². The bacteria *Escherichia coli* are commensal organisms (an organism that lives in close association with another without causing harm) in the gastrointestinal tract of both children and adults. This characteristic makes them useful for investigating the prevalence of antibiotic resistance in commensal bacteria^{1,14}. The normal flora has a significant contribution towards the emergence of antimicrobial resistance of infected microorganisms during the last few years. There is growing evidence from developing countries that studies on the normal flora indicate that antimicrobial resistance rates of diverse antimicrobial agents are higher than expected¹. In addition to the amount of evidence for resistance of the normal flora against these agents, there is also substantial evidence that these agents have an established role in causing infectious diseases globally. Each year in developing countries, there are 2.2 million deaths due to diarrhoea; approximately 130-175 million cases of uncomplicated urinary tract infection (UTI) globally occur, with >80% of these cases caused by *Escherichia coli*¹¹.

Escherichia coli is also known to be a major cause of gastrointestinal tract infections extending to extra-gastrointestinal sites, including blood, urinary tract and central nervous system^{2,24}. *Escherichia coli* is a primary cause of illnesses and deaths around the globe. The

emergence of antimicrobial-resistant strains makes treatment of *E. coli* infections more difficult. Multiple strains of *E. coli* are becoming increasingly resistant to standard antibiotics in many parts of the world¹⁶. The growing problems associated with bacterial antibiotic resistance are coming from many sources and reasons. Some common contributing factors to the bacterial resistance problem include the careless use of antibiotics, dosing errors, and missing doses while treating people and some animals. Multidrug-resistant (MDR) organisms are becoming more frequently observed in the general population, as well as in hospitals and healthcare settings, before being discharged home from the hospital⁹. The prevalence of Extended-spectrum β -lactamase (ESBL) bacterial isolates differs by geographic area, as seen by the low levels reported in Japan, Sweden, and Singapore (3-8%) versus the much greater rates reported in studies conducted on other continents. Extended-spectrum β -lactamase (ESBL) production is a significant means of *E. coli* resistance⁶. In 1999, it was first identified in an isolate from India and consequently became widespread worldwide. Carbapenems are the most powerful tools against Gram-negative bacilli. Due to the extensive misuse of this antibiotic, Carbapenem resistance has also developed in *Escherichia coli*. Bacteria resistant to carbapenems have the potential to spread rapidly within the hospital environment and across continents. Timely detection can help in appropriate and aggressive infection control measures, and an effective antibiotic policy can help in confronting the menace of antibiotic resistance¹⁵. Carbapenem-resistant *E. Coli* is of particular significance since these drugs represent one of the last remaining classes of effective antimicrobial therapy against this organism. NDM and OXA48-like carbapenemases are among the most frequently identified carbapenemases in *E. coli*, with overall prevalence rates of 38% to 92% and 15% to 26%, respectively⁸.

There is an emerging public health threat posed by increasing antimicrobial resistance in Gram-negative bacteria, i.e. *E. coli*. In our country, this necessitates ongoing surveillance of antimicrobial resistance trends in our area.

Materials & Methods:

The study was carried out to detect carbapenem resistance in *Escherichia coli* isolates in the Microbiology Department at Keral Verma Subharti College of Science, Meerut (U.P) and clinical samples were collected from the patients attending Subharti Medical College & Hospital, Meerut (U.P). A total of 206 *E.coli* isolates were included for the detection of Carbapenem resistance in different types of specimens, such as Urine, Blood, and Pus. The protocol of the study was reviewed and approved by the Research & Development Cell of Swami Vivekanand Subharti University, Meerut (U.P.).

Inclusion criteria

E. coli isolates from patient specimens were obtained from both the Intensive Care Unit (ICU) and from hospital wards. Specimens included urine, blood and pus.

Exclusion criteria

Clinical samples with polymicrobial growth =3 types of organisms.

Collection of bacterial isolates

The collection of clinical samples from patients in the ICU and ward was done according to the accepted guidelines and was not repeated. The clinical samples collected were cultured on Mac Conkey agar, Cysteine Lactose Electrolyte Deficient (CLED) and Blood agar (Merck, Germany) and incubated for 24 hours at 37 degrees Celsius. Colonies that produced fermentation of lactose on both Mac Conkey agar and CLED were identified by their morphology as circular rose-pink to red colonies on MacConkey and yellow colonies on CLED. Identification of colonies of *Escherichia coli* was done by Gram staining, morphology and through biochemical testing process for confirmation.

Carbapenem resistance detection in *E. coli*

The Clinical and Laboratory Standards Institute guidelines were used for the routine antibiotic susceptibility testing. Preparation of a suspension of isolate *E. coli* was used to adjust the turbidity to equal 0.5 McFarland standards. This suspension inoculates Mueller-Hinton agar. All confirmed *E. coli* (n=206) was tested for

carbapenem resistance using discs of imipenem and meropenem (10 ig). We considered an isolate to be resistant when its zone of inhibition was 19 mm or less. All the tests were performed on Mueller-Hinton agar according to Center for Disease Control (CDC) and Clinical and Laboratory Standards Institute (CLSI) guidelines. A lawn culture was prepared on Mueller-Hinton agar (MHA) using an overnight culture of *E. coli* (ATCC 25922) that was adjusted to 0.5 McFarland's standards. After 15 minutes of drying, a 10 ig meropenem disc was placed in the centre of the plate. Then, each isolate was streaked from the edge of the disc to the edge of the plate. The zone of inhibition was measured (in mm from the edge of the disc after an overnight incubation at 37° C) using CLSI guidelines.



Figure 1. Disk diffusion assay performed on Mueller-Hinton Agar (MHA) for representative multidrug-resistant Gram-negative infections. Observation of Carbapenem discs: Imipenem (IPM 10 µg), Meropenem (MRP 10 µg), and Ertapenem (ETP 10 µg) for treating Carbapenem-Resistant *Escherichia coli*.

Carbapenem resistance detection by Automated method: Further evaluation of the isolates of *Escherichia coli* for susceptibility to carbapenems was conducted using Vitek-2 (Biomérieux, Durham, U.S.A.). Testing was done utilizing the AST 405 card for antibiotics against gram-negative bacilli, where the following antibiotics could be tested for susceptibility: ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefalotin, cefoxitin, cefixime, ceftazidime, ceftriaxone, ertapenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, nitrofurantoin, co-trimoxazole, and imipenem, meropenem and ertapenem. To inoculate a GN405 card, the standardised inoculum must achieve turbidity corresponding to a McFarland turbidity range of 0.50 to 0.63.

Identification and antimicrobial susceptibility testing were also performed using the vitek-2 Compact system in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI).

The antimicrobial susceptibility profile was obtained from the VITEK 2 system, which demonstrated elevated minimum inhibitory concentration (MIC) values for carbapenems, with imipenem (=16 µg/mL), meropenem (=16 µg/mL), and ertapenem (=8 µg/mL), all interpreted as resistant according to CLSI. These findings confirm the presence of carbapenem-resistant isolates.

The bacterial suspension was first prepared and standardised using a Densi CHEK Plus instrument, where the barcode reader automatically scanned the test kit barcode. This barcode was then linked to the corresponding sample ID in the software, eliminating the need for manual labelling and allowing alphanumeric entries of up to 20 digits. The test kits were subsequently filled and sealed by placing the cassettes into the Filler Station, after which they were transferred to the Load/Unload Station for automatic sealing. The sealed test kits were then moved into the reader/incubator system, where incubation and analysis were carried out automatically. Upon completion, the test kits were ejected into the waste collection bin, and the used cassettes were disposed of after returning to the Load/Unload Station.

Results

The present study analysed a total of 630 clinical samples. Out of 630 clinical samples, 206 isolates exhibited significant growth of Gram-negative *E. coli*, comprising urine (n = 146), blood (n = 42), and pus (n = 18). Among these, 206 isolates (32.69%) were identified as *Escherichia coli*, indicating a substantial burden of Gram-negative bacterial infections across the sampled clinical specimens and were further evaluated for carbapenem-resistance to determine the prevalence and antimicrobial resistance profile of *Escherichia coli*.

A sample-wise distribution of *E. coli* isolates revealed that urine samples contributed the highest proportion (n = 146; 70.87%), followed by blood samples (n = 42; 20.38%), and pus samples (n

= 18; 8.73%). This distribution underscores the predominance of *E. coli* in urinary tract infections, while also highlighting its significant involvement in bloodstream and wound-associated infections. Figure 2 provides a thorough explanation of how *E. Coli* isolates were distributed among various clinical samples in the current investigation.

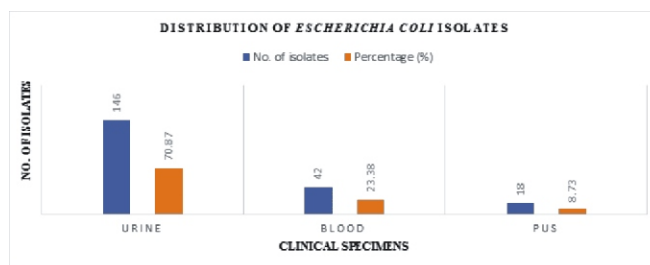


Figure 2. Comparative Distribution of *Escherichia coli* isolates by different clinical specimens

A total of 206 *E. coli* isolates included 58 (28.15%) male patients and 148 (71.84%) female patients (see Table 2). Among 206 *E. coli* isolates, 64 (31.06%) were carbapenem resistant and 142 (68.93%) demonstrated susceptibility to carbapenems (see Table 3).

Table 2. Gender wise distribution of *Escherichia coli* isolates (n=206)

Gender	Total No. of Cases	Percentage (%)
Male	58	28.15
Female	148	71.84
Total	206	100

Table 3. Detection of Carbapenem-Resistant *Escherichia coli*.

Category	No. of <i>E.coli</i> (Percentage %)
Carbapenem-Resistant <i>E.coli</i>	64 (31.06%)
Carbapenem-Sensitive <i>E.coli</i>	142 (68.93%)

Discussion

The rise of antibiotic resistance and its rapid spread is a major problem for global health, particularly within hospitals, where there is an enormous amount of selective pressure placed upon the population to develop an antibiotic-resistant strain of bacteria. As bacteria develop this resistance, the resulting strains of antibiotic-resistant bacteria are capable of causing serious or fatal infections that are much more difficult to treat than traditional infections. This results in an increase in mortality and morbidity rates^{17,21}. The incremental rise in the rate of resistance in gram-negative enteric organisms

exemplifies the ability of these organisms to continue to acquire, retain and express multiple forms of genetic resistance; this ability occurs especially among the members of Enterobacteriaceae and the other gram-negative organisms as well. Several mechanisms of resistance are mediated by mobile genetic elements (e.g., plasmids, transposons, integrons), which allow for the presence of multiple classes of antibiotic resistance and the movement of antibiotic-resistant genes between entire populations of bacteria via horizontal gene transfer⁵. The emergence and prevalence of antibiotic-resistant organisms provide antibiotic resistance in gram-negative pathogens as a primary therapeutic and epidemiologic challenge, which requires strict implementation of infection prevention and control procedures. To reduce the incidence of dissemination of resistant strains of bacteria within healthcare environments and to preserve the efficacy of existing antimicrobial products, effective strategies for treatment and prevention of antibiotic-resistant infections need to be implemented^{5,7}.

Currently, Carbapenem-resistant *Escherichia coli* is one of the main pathogens among Carbapenem-resistant Enterobacteriaceae, causing various clinical infections^{12, 13}. This finding is in agreement with certain previous investigations that have reported comparatively lower prevalence rates, while it is notably lower than other studies from different regions of India that have documented a higher frequency of *E. coli* isolation. Such discrepancies in prevalence may be attributed to variations in study design, sample size, patient population, and types of clinical specimens analysed. Furthermore, the observed geographic variability in the distribution of *E. coli* highlights the significant role of regional epidemiological factors, including differences in healthcare infrastructure, infection control practices, antibiotic usage patterns, and local microbial ecology. Environmental conditions and community-level transmission dynamics may also contribute to these variations. Therefore, the prevalence of *E. coli* cannot be generalised across regions, emphasising the need for continuous local surveillance to better understand its epidemiology and to guide appropriate clinical and antimicrobial stewardship strategies^{18,20}.

In the present study, a significant majority of the patients from which we extracted isolates were females, representing an incidence rate of 71.84%. Consequently, based on the predominant incidence rates among females, it appears that females are at a higher risk of infection with the source organisms than males. While this number demonstrates female patients have a higher incidence rate of morbidity due to the isolated pathogens, it should not be interpreted as a true representation of the epidemiological status of these organisms within their respective contexts. The impact of the bias involved with selecting a single clinical area for specimen collection is very likely to greatly modify the gender distribution for isolates collected in the specified timeframe, and therefore should be taken into account when reviewing the epidemiologic data presented in this report. Several investigations have reported a higher prevalence of infections among female patients, particularly in cases involving urinary tract infections, where anatomical and physiological factors predispose females to increased susceptibility. In the present context, although a greater proportion of female cases was observed, previous studies have shown variability in gender distribution. It was reported that females accounted for over half of the total cases in their study population, while males constituted a comparatively lower percentage, highlighting the inconsistency in gender-based prevalence across different settings¹⁰.

On the other hand, there is some research showing that there is a stronger female sample; in most of these studies, the number of female patients is classified as moderate to high. The differences in gender from study to study have been attributed to an interplay of many complex biological, behavioural, and environmental influences Shah. In addition to the fact that there are differences in the manner in which men and women seek health care, it is possible that this may have an impact on differences in diagnosis and reporting of infections, with women perhaps waiting longer before seeking medical attention or being diagnosed with an advanced infection. In addition, comorbidity, lifestyle and immunity levels may influence male/female susceptibility to infection differently. Gender has a significant effect on the epidemiology of infection, with all

studies emphasising the pronounced effect of gender on infections. The authors state that the overall number of infections is greater amongst females in their research; therefore, gender should be considered an important epidemiological variable in infectious disease research³.

In the present study, out of a total of 206 *Escherichia coli* isolates, 64 (31.06%) were identified as carbapenem-resistant *E. coli* (CREC). This rate of prevalence appears similar to rates found in previous studies from multiple geographical areas that have shown moderate rates of carbapenem resistance among *E. coli* isolates. Some studies have reported higher prevalence rates than those found in this study, while other studies have reported lower rates¹⁷. Additionally, the increasing prevalence of carbapenem-resistant *E. coli* continues to pose a significant clinical problem as it limits treatment options and complicates the management of potentially fatal infections¹³. The results from this study highlight the need for continuous surveillance, rational use of antibiotics, and aggressive infection-control practices to reduce the transmission of carbapenem-resistant bacteria in all areas and practice settings¹⁹. These findings show inconsistency in the frequency of the emergence of carbapenemase-producing organisms in the various studies and multiple hospitals included in the analyses performed for this article. Variability in carbapenemase-producing organism occurrence may be attributed to numerous factors influencing the emergence and spread of resistance to antimicrobials among clinical infections in Gram-negative bacteria.

Conclusion

Carbapenem-Resistant *E. coli* (CREC) is one of the most significant and fastest-growing threats to public health today, impacting the treatment of bacterial infections. The increase in the prevalence of CREC strains presents major challenges for controlling these infections and decreasing the efficacy of therapeutic strategies in treating these and many other infections by limiting treatment options for patients. For this to occur, ongoing discovery and surveillance of changing patterns of antimicrobial resistance (AMR) will be necessary

in order to contain the spread of resistant microorganisms and to inform the use of AmT to optimise the outcomes for patients through evidence-based treatment strategies. Carbapenem-resistant *E. coli* make it very difficult to select the most appropriate class of antimicrobial agents, resulting in higher levels of morbidity and mortality, as well as increased healthcare costs. Therefore, the prompt identification of carbapenem-resistant *E. coli* is crucial for optimising the clinical outcome for the patient and developing effective IPC strategies for preventing the transmission of these resistant organisms to other patients. Preventing the creation and spread of carbapenem-resistant organisms in both hospital and healthcare system settings requires a multifaceted approach involving coordinated IPC practices, judicious use of antibiotics through antibiotic stewardship programs, and establishing a strong surveillance system for antimicrobial resistance. Use of additional strategy options, including antibiotic rotation and regular policy review interventions, may be necessary to mitigate the creation of selective pressure on bacteria, causing increased rates of antimicrobial resistance.

Clinical microbiology labs must routinely monitor for the emergence of carbapenems, document their results accurately (consistently), and report them on time to provide information to physicians and to assist public health efforts to contain the spread of multidrug-resistant organisms.

Conflict of interest

The authors have no Conflict of interest regarding this investigation.

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Ethical clearance

Approval from the Department of Microbiology, Keral Verma Subharti College of Science and Subharti Medical College & Hospital, Meerut (U.P) (Ref. No. SVSU/Asst. Reg./2024/x-2410) was obtained before study commencement.

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