INNOVATIONS AND CHALLENGES IN PLANT AND ANIMAL SCIENCES

Proceedings of the 1st International Conference (RTPAS-2025)

RTPAS 2025 GDC Budaun

Editors:
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Dr. Gaurav Kumar Singh
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Ph.D. (Botany), Aligarh Muslim University
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Dr. Priyanka Singh specializes in plant physiology and stress biology, with 19 SCI-indexed publications and 2,220+ citations. She has received multiple awards and convened the international conference RTPAS-2025.

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Dr. Gaurav Kumar Singh specializes in nematode taxonomy, biodiversity, and biocontrol, with SCI-indexed publications and extensive teaching and research experience. He also served as Convenor of RTPAS-2025, contributing to its academic success.

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Dr. Sachin Kumar has published 29 SCI-indexed papers with 595+ citations (h-index 16, i10-index 22), contributing significantly to mathematical sciences. As Coordinator of RTPAS-2025, he ensured the smooth execution of the international conference.

Assistant Professor

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INNOVATIONS AND CHALLENGES IN PLANT AND ANIMAL SCIENCES: Proceedings of the 1st International Conference (RTPAS-2025)

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CHIEF GUEST'S ADDRESS

Hon'ble Dharmpal Singh
Minister of Animal Husbandry & Dairying
Government of Uttar Pradesh

I am pleased to extend my sincere congratulations to the organizing committee of the *1st International Conference on Recent Trends in Plant and Animal Sciences (RTPAS-2025)*, led by the young and dedicated team of **Dr. Priyanka Singh, Dr. Sachin Kumar,** and **Dr. Gaurav Kumar Singh**. This prestigious international event is a proud milestone for the **Government Degree College, Budaun**, and a testament to its growing stature in the academic and research community.

As Minister of Animal Husbandry & Dairying, I especially appreciate the conference's focus on themes of direct relevance to animal health, sustainable livestock management, biodiversity conservation, and innovative technologies in the field of animal sciences.

The gathering of eminent experts, researchers, and students from India and abroad provides a valuable platform for exchanging ideas, fostering collaborations, and developing solutions that will benefit both the scientific community and society at large.

I commend the commitment, vision, and meticulous planning demonstrated by the organizers in hosting a conference of such scale and importance. I am confident that the deliberations will inspire fresh perspectives, advance research, and contribute meaningfully to academic progress and societal welfare.

I wish RTPAS-2025 every success and trust that it will leave a lasting legacy for future academic endeavors at GDC Budaun.

With best wishes,

Dharmpal Singh
Minister of Animal Husbandry & Dairying
Government of Uttar Pradesh

MESSAGE

Prof. (Dr.) Shashi Kapoor
Joint Director, Higher Education
Uttar Pradesh

I am pleased to extend my warm greetings and congratulations to the young and dynamic organizing team—**Dr. Priyanka Singh, Dr. Sachin Kumar,** and **Dr. Gaurav Kumar Singh**—for their commendable efforts in hosting the *1st International Conference on Recent Trends in Plant and Animal Sciences (RTPAS-2025)* at **Government Degree College, Budaun**. This prestigious international gathering is indeed a proud moment for the entire GDC family, showcasing the institution's dedication to academic growth, research excellence, and global collaboration. I wish to place on record my sincere appreciation for the leadership and unwavering support of **Principal Dr. Shraddha Gupta**, whose guidance has been instrumental in making this event a reality.

As Joint Director of Higher Education, I strongly believe in the importance of platforms that encourage meaningful academic exchange, foster interdisciplinary collaboration, and inspire young minds to engage with contemporary scientific challenges. RTPAS-2025.

I commend the **GDC Budaun team** for their vision and perseverance in bringing together distinguished scientists, educators, and students from across the world. Such initiatives not only enhance the academic reputation of the college but also contribute significantly to building a culture of innovation and research excellence in Uttar Pradesh.

I wish the conference grand success and hope that it becomes a catalyst for continued research collaborations, groundbreaking ideas, and lasting professional relationships.

With best wishes,

Prof. (Dr.) Shashi Kapoor

Prof. (Dr.) Shashi Kapoor
Joint Director, Higher Education
Uttar Pradesh

MESSAGE FROM THE REGIONAL HIGHER EDUCATION OFFICER, BAREILLY

Prof. (Dr.) Sudhir Kumar
Regional Higher Education Officer, Bareilly,
Uttar Pradesh

It gives me immense pleasure to convey my warm greetings and best wishes to the organizers of the 1st International Conference on Recent Trends in Plant and Animal Sciences (RTPAS-2025) being held at Government Degree College, Budaun. This event marks a significant milestone in the academic journey of the institution and reflects its growing commitment to excellence in teaching, research, and global collaboration.

I deeply appreciate the sincere efforts of the organizing team—**Dr. Priyanka Singh, Dr. Sachin Kumar,** and **Dr. Gaurav Kumar Singh**—whose vision and dedication have transformed this idea into a reality. My special appreciation also goes to Principal Dr. Shraddha Gupta, whose leadership and encouragement have been instrumental in nurturing such a scholarly culture within the college.

As Regional Higher Education Officer, Bareilly, I firmly believe that such international platforms create opportunities to exchange knowledge, encourage interdisciplinary dialogue, and motivate young researchers to contribute meaningfully to society's scientific and educational progress. The RTPAS-2025, by bringing together distinguished scientists, educators, and students on a single platform, truly embodies this spirit of academic enrichment.

I am confident that this conference will not only broaden intellectual horizons but also open new avenues of collaboration, innovation, and research excellence. May it inspire every participant to contribute toward building a brighter and more knowledge-driven future.

With sincere best wishes for the resounding success of the conference.

Prof. Sudhir Kumar

Regional Higher Education Officer, Bareilly

Uttar Pradesh

PRINCIPAL'S MESSAGE: GUIDING THE SPIRIT OF RTPAS - 2025

Dr. Shraddha Gupta
Principal,
Government Degree College, Budaun
Uttar Pradesh

It gives me immense pride and satisfaction to extend my heartfelt best wishes on the occasion of the *1st International Conference on Recent Trends in Plant and Animal Sciences (RTPAS-2025)*, organized by our college. This historic academic event marks not only a milestone for our institution but also a celebration of intellectual exchange across nations and disciplines.

Science flourishes when minds meet, when ideas are tested, and when knowledge is shared selflessly. By bringing together eminent scholars, researchers, and young academicians from India and abroad, this conference exemplifies our shared commitment to advancing scientific inquiry and addressing challenges that matter to humanity—be it sustainability, biodiversity, environmental balance, or innovation in plant and animal sciences.

I firmly believe that the discussions and deliberations during this conference will ignite new collaborations, inspire future research, and ultimately contribute to the betterment of society at large. Our institution feels honored to host such a vibrant forum, and I am deeply appreciative of the tireless efforts of the organizing team in making this vision a reality.

As Principal, I extend my best wishes to every participant. May RTPAS-2025 become a beacon of knowledge and an enduring symbol of academic excellence, motivating us to continue this journey of discovery with even greater zeal in the years ahead.

With warm regards and best wishes,

Shraddha

Dr. Shraddha Gupta
Principal
Government Degree College, Budaun

MESSAGE FROM THE CONVENOR

Dr. Priyanka Singh
Convenor, RTPAS-2025
Government Degree College, Budaun

It gives me immense pleasure to extend a heartfelt welcome to all distinguished speakers, participants, and delegates from across the globe to the *1st International Conference on Recent Trends in Plant and Animal Sciences (RTPAS-2025)*, jointly organized by the Departments of Botany and Zoology, Government Degree College, Budaun.

This conference marks a significant step forward in our collective pursuit of knowledge, bringing together a vibrant community of researchers, academicians, and young scholars. The multidisciplinary themes of this event—spanning from biodiversity conservation, nanotechnology, and soil health, to animal sciences, climate resilience, and sustainable agriculture—reflect the urgent need to address today's global scientific challenges with holistic and innovative approaches.

We are deeply honoured by the presence of eminent keynote speakers, distinguished session chairs, and enthusiastic participants, whose intellectual contributions from the cornerstone of this academic endeavour. Your research, insights, and discussions not only enrich the scientific community but also inspire future collaborations across disciplines and borders.

I sincerely hope that this Book serves not only as a record of your invaluable contributions but also as a lasting resource for continued learning, dialogue, and discovery.

On behalf of the organizing committee, I extend my gratitude to our esteemed Principal, colleagues, and dedicated team members, whose constant support made this event possible. I also express my appreciation to all participants whose active involvement transforms this conference into a true celebration of science and scholarship.

Let us work together to transform ideas into impactful actions for a sustainable and better future.

With warm regards and best wishes,

Dr. Priyanka Singh

Convenor, RTPAS-2025

MESSAGE FROM THE CONVENOR

Dr. Gaurav Kumar Singh
Convenor, RTPAS-2025
Government Degree College, Budaun

It is with great pride and joy that I welcome all participants, keynote speakers, and delegates to the *1st International Conference on Recent Trends in Plant and Animal Sciences (RTPAS-2025)*. This academic gathering stands as a testament to our collective commitment to advancing science, fostering innovation, and nurturing young minds.

The themes addressed in this conference—ranging from taxonomy and biodiversity to environmental sustainability and modern technological interventions—highlight the dynamic progress in biological sciences and their direct relevance to society. Bringing together such a wide spectrum of expertise on one platform is itself a remarkable achievement, and I am confident that the knowledge exchanged here will pave the way for future collaborations and scientific breakthroughs.

This Book reflects the breadth and depth of research contributions shared during the conference. I encourage all readers to explore its pages with curiosity and openness, as it represents not only individual scholarship but also the spirit of collective learning.

On behalf of the organizing committee, I express my sincere gratitude to all the distinguished guests, speakers, participants, and my fellow team members for their wholehearted support. Together, we have shaped RTPAS-2025 into a vibrant forum for discussion, inspiration, and progress.

I look forward to seeing the seeds of ideas sown here grow into fruitful outcomes for science and society alike.

With warm regards and best wishes,

Gaurav

Dr. Gaurav Kumar Singh

Convenor, RTPAS-2025

MESSAGE FROM THE COORDINATOR

Dr. Sachin Kumar
Coordinator, RTPAS-2025
Government Degree College, Budaun

It is with immense pride and joy that I extend my warm greetings to all the participants, distinguished speakers, and delegates of the 1st International Conference on Recent Trends in Plant and Animal Sciences (RTPAS-2025), jointly organized by the Departments of Botany and Zoology, Government Degree College, Budaun, Uttar Pradesh, India.

This conference is not merely an event—it is a vision realized. It brings together scientists, academicians, research scholars, and students from across the globe to share their knowledge, exchange ideas, and build collaborations for the advancement of science and society.

This Book is a reflection of the intellectual diversity of this conference. It encompasses a wide spectrum of themes, including plant and animal sciences, veterinary research, biotechnology, nanotechnology, environmental management, biodiversity conservation, sustainable agriculture, and human health sciences. Each chapter represents the hard work, dedication, and passion of researchers committed to pushing the frontiers of knowledge.

As the Coordinator, I feel deeply honoured to have been part of this academic journey. I sincerely thank our respected Principal, **Dr. Shraddha Gupta**, for her constant encouragement and guidance; our Conveners, **Dr. Priyanka Singh** and **Dr. Gaurav Kumar Singh**, for their leadership and dedication whose tireless efforts made this international event a reality.

I hope that the deliberations and interactions during RTPAS-2025 will inspire new ideas, foster meaningful collaborations, and contribute towards sustainable solutions for the challenges faced by humanity and the environment.

With best wishes for fruitful learning and collaboration.

Dr. Sachin Kumar

Coordinator, RTPAS-2025

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1. Indian Spice Essential Oil: A Promising Green And Eco-Friendly Food Preservative

Dr. Amrita yadav[1]*, Mr. Ram Prakash Pal[2]

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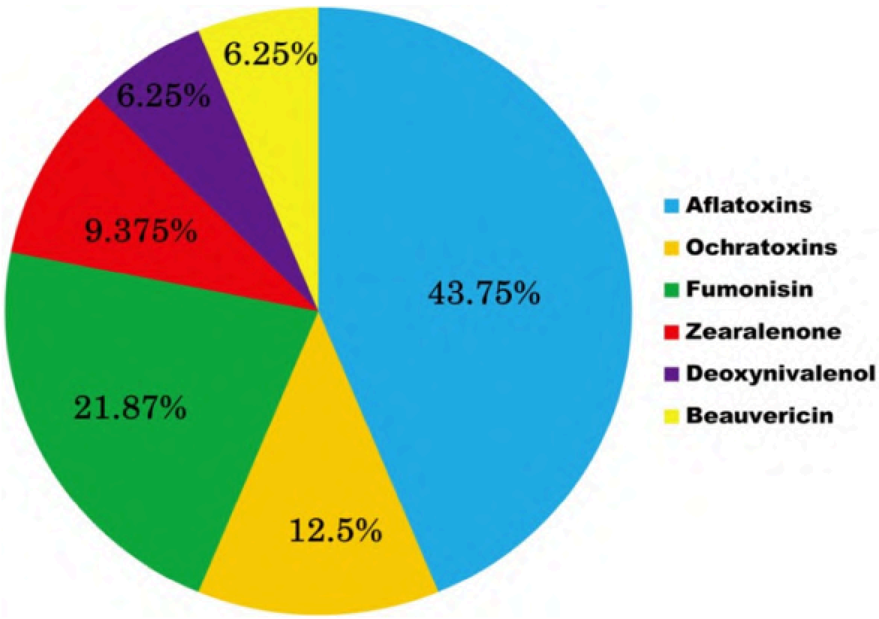
Abstract

Aflatoxin produced by *Aspergillus flavus* is a liver toxic (hepatotoxic) carcinogenic, mutagenic, difuran-containing, polyketide- derived food contaminants. The edible items containing aflatoxin concentrations of 1 mg/kg or higher doses has been suspected to cause aflatoxicosis. Stored food commodities viz., legumes wheat, etc are prone to molds and aflatoxin contamination. IARC (International Agency for Research on Cancer) has been classified Aflatoxin B1 as class-1 human carcinogenic agents. Therefore, the regulatory authorities related to food safety have established strict regulations on trading process, storage techniques and monitoring of food to reduce the lowering health risk caused by aflatoxin B1. Nowadays, application of plant derived phytochemicals such as essential oils possesses stronrisk caused potential in the management of food borne molds and mycotoxin contamination. The present study revealed the antifungal and preservative potential of nanoencapsulated mace essential oil with probable mode of action. Free and nanoencapsulated exhibited strong Antifungal and antiaflatoxigenic activity with minimum inhibitory concentration and minimum aflatoxin inhibitory concentration of 2.0 μL/mL and 1.7μL/mL respectively. The finding could be great significance in inhibiting the growth and Aflatoxin produced by *A. flavus* in stored food commodities.

Keywords: Aflatoxin, Mace essential oil, Nanoencapsulation, Food preservative.

1. Introduction

Stored food commodities such as legume seeds viz., cowpea, chickpea, green gram, garden pea, black gram, lentil and pigeon pea and their processed goods are vulnerable to biodeterioration due to the invasion of microorganisms by various storage pests including insects and food borne fungi from the time of harvest to consumption i.e., "POSTHARVEST LOSSES". Among all Mycotoxin, Aflatoxin contamination is one of the major problems of stored food commodities. Mycotoxins are toxic secondary metabolites synthesised by toxigenic fungus species viz., *Aspergillus, fusarium*, *penicillium,* etc. After using directly or indirectly mycotoxin, contaminated food and food product may causes various health issues on both human being and livestock. Literature studies confirms the presence of more than 200 different types mycotoxins in nature viz., citrinin , sterigmatocystin zearalenone, cyclopiazonic acid, Aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), fumonisins B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3), etc., But it is found that, aflatoxin B_1(AFB_1), contamination has been received considerable attention of various food industries and regulatory authority because (AFB_1), shows its carcinogenicity, genotoxicity, hepatotoxicity, oestrogenicity, reproductive disorders, immunosuppression effect depending on severity and duration of exposure.



Many available food safety means and novel storage methods for moulds contamination and its mycotoxin degradation along with oxidative decay in various areas of the world remain a major challenge. Various techniques such as modified atmosphere packing (MAP), sorting technology, electromagnetic radiation, Low temperature, cooling, airing, fast drying of vacuum packaging, approaches do not apply dramatically in broad scales to the bulk storage of foodstuffs. Nowadays, consumer awareness towards green consumerism and development of resistance against the synthetic preservatives declines its uses. Thus, there is an urgent need of some safer methods to manage the AFB_1 contamination in various food and food product. Since ancient times, plant and its products have been recognised for their ability to enhance human health and lifestyle

(Zhang *et al*., 2023). Higher plants provide a large number of primary and secondary metabolites which possess various green chemical and ability to have fight against the micro-organism. Among many natural products obtained from the plant. Plant essential oils (EOs) and their phytochemicals (terpenes, terpenoids, phenylpropenes, aldehydes, ketones, ethers, and phenolics) are good sources of food additives and are commonly adopted by consumers and industry due to its strong antimicrobial properties. (Nychas 1995, Prakash *et al*., 2018). But due to the volatilization nature or early degradation limits its defence mechanism and make it less effective in various food system. Encapsulation provides packing and protecting of the various phytochemicals present in essential oil within a naturally derived wall material with increased stability and controlled release in the food system (wang *et al*.,2024). Among many green products, Indian spices such as mace also known as dry nutmeg fruit (*Myristica fragrans Houtt.*) covered with orange lacy aril is rich in various phytochemicals and have many medicinal use. (Kulandhaivel *et al*., 2011). Literature studies confirmed the *Myristica fragrans* have strong antioxidant and antimicrobial activity (Singh, Marimuthu, Murali, & Bawa, 2005, kaur *et al*.., 2024). The mace essential oil (MEO) has previously been reported to possess antibacterial, antihyperlipidemic, anti-inflammtory and antifungal activity (Ali *et al*.,2021).

Fig.1: Dry spice mace (*Myristica fragrans)*

0*Houtt.)* c *fragransFig.,*

2. Material and methods

2.1 Mycoflora analysis on selected legume seed

Commonly used legumes seed such as soyabean*Glycine max* (L.) , lobiya (*Vigna unguiculata* (L.) Walp)*,), moongVigna radiate* (L.) Wilczek) *, Vigna mungo* (L.) Hepper, toor daal *(Cajanas cajan* (L.) Millsp)*,* matar *(Cicer arietinum* (L)), *Merrill* were selected for the mycoflora analysis were stored at 4°C till experimental use . Thereafter, legume seeds were placed in petriplates 12 mL PDA medium. One ml of spore suspension was poured in petriplates . All the petriplate were kept in the BOD incubator. After the incubation period growth of colonies were counted. The % appearance frequency was obtained from the given equation.

$$(\%) appearance\ frequency = (Total\ no{:}\ of\ isolates\ of\ each\ fungus\ )/(\ total\ fungus\ isolates$$
$$\times 100$$

2.2 Isolation of mace essential oil

Mace essential oil was extracted from clean and fresh dry fruit through hydrodistillation process using a Clevenger's type apparatus for 3 to 5 hours.

2.3 Nanoencapsulation of mace essential oil

The nanoencapsulation of mace essential oil was prepared using chitosan, cinnamic acid and EDC according to the procedure of Beyki *et al*., 2014 with slight modification. After formation nanoencapsulated mace essential oil was characterized using SEM.

2.4 Determination of antimicrobial activity

The antifungal and aflatoxin B_1 inhibitory activity of both free and nanoencapsulated essential oil was done using SMKY medium Prakash *et al*., 2010. Requisite amounts test essential oil were dissolved in 0.5 mL Tween-20 (1%) and then added separately to 24.5 mL SMKY liquid medium. 40 µL spore suspension of the toxigenic strain of *A. flavus* was inoculated into each conical flask and kept in B.O.D incubators for 11-10-day. The lowest concentration causes complete inhibition of fungal growth was considered as Minimum inhibitory concentration.

2.5 Free radical scavenging activity

The antioxidant activity of both free and nano encapsulated mace essential oil was determined by measuring the level of bleaching of purple colour methanolic solution of DPPH to pale yellow colour following Prakash *et al*., 2012. Different concentration of test essential oil were added to 5 mL of 0.004% methanolic solution of DPPH and placed in light free area for half an hour. After 30 minutes, the absorbance was measured at

517 nm against blank. The % inhibition of DPPH free radical with respect to blank was calculated using the following derived equation.

$$\% \text{ Inhibition} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where, A_{blank} is the absorbance of the sample that possesses all reagents, and A_{sample} is the absorbance of the sample to be analysed.

2.6 Mechanism of action of nanoencapsulated mace essential oil against A. flavus

2.6.1 Efficacy of nanoencapsulated mace essential oil on ergosterol content in plasma membrane of fungus

The effect of different doses of nanoencapsulated mace essential oil on ergosterol content in the plasma membrane of A. *flavus*) was measured following Tian *et al*., 2012

$$\% \text{ Ergosterol} = A\ (\% \text{ ergosterol} + \% \text{ 24(28) dehydroergosterol}) - B\ (\% \text{ 24(28) dehydroergosterol})$$

$$A\ (\% \text{ ergosterol} + \% \text{ 24(28) dehydroergosterol}) = (\text{Abs.}282/290)/ \text{ pellet weight}$$

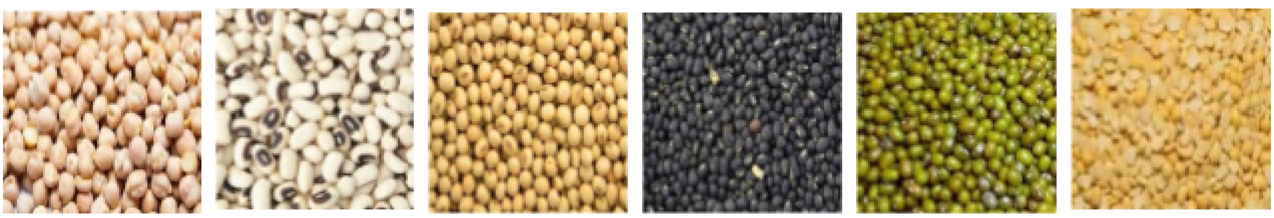
$$B\ (\% \text{ 24(28) dehydroergosterol}) = (\text{Abs.}230/518)/\text{pellet weight;}$$

Where, 290 and 518 are the E values (in percentages per cm) determined for crystalline ergosterol and 24(28) dehydroergosterol, respectively, and pellet weight is the net wet weight (g).

3. Results and discussion

3.1. Mycoflora analysis from the selected legume seed

Results obtained from Mycoflora analysis shows that legumes seed samples were contaminated with many food borne molds which degrade its quality and quantity. Study also suggest that selected legume seed are also contaminated with toxigenic species of *Aspergillus flavus* and other food borne fungus.



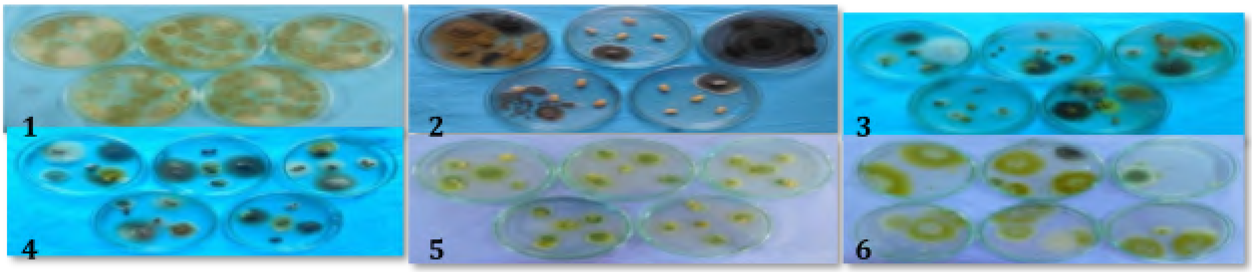


Fig.2 Legume seed and Mycoflora Analysis.

3.2. Isolation of various phytochemicals from Mace essential oil (MEO)

For the isolation of essential oil from mace, aril (as a third integuments) of the *Myrisitca fragranse* was used and MEO was isolated using Clevenger-type hydrodistillation method during which the plant materials is processed with steam. As the plant tissue breakdown, the essential oils and water vapour are released, which condendesed and cooled and isolated through the separators. All the isolated phytochemicals has been identified and quantified by GCMS. Myristicne, methyl eugenol and gama terinene etc was recognised as a major compound which have strong antimicrobial properties.

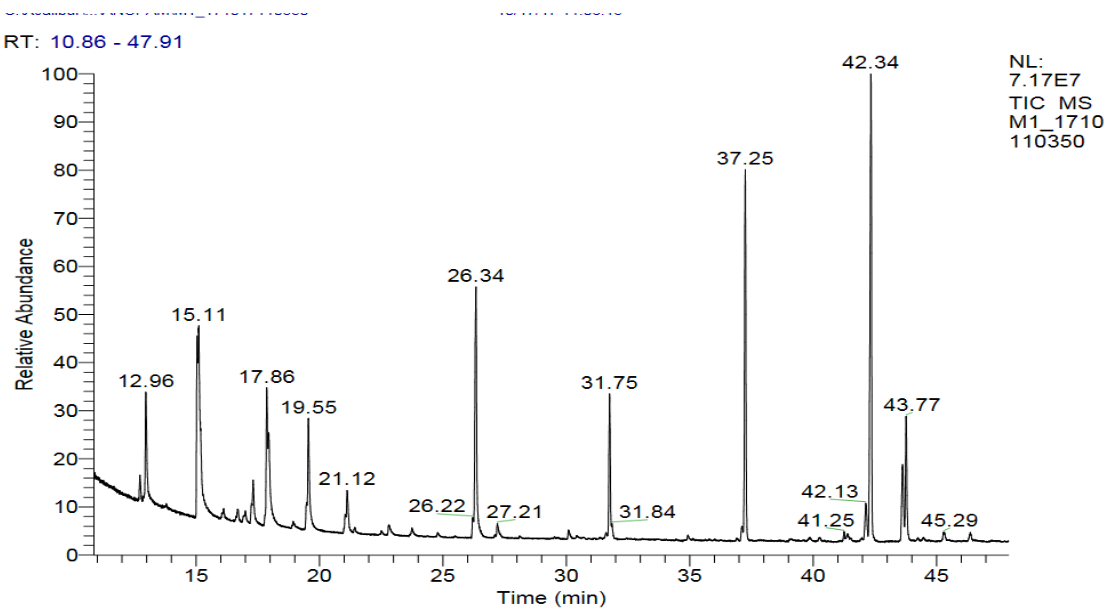
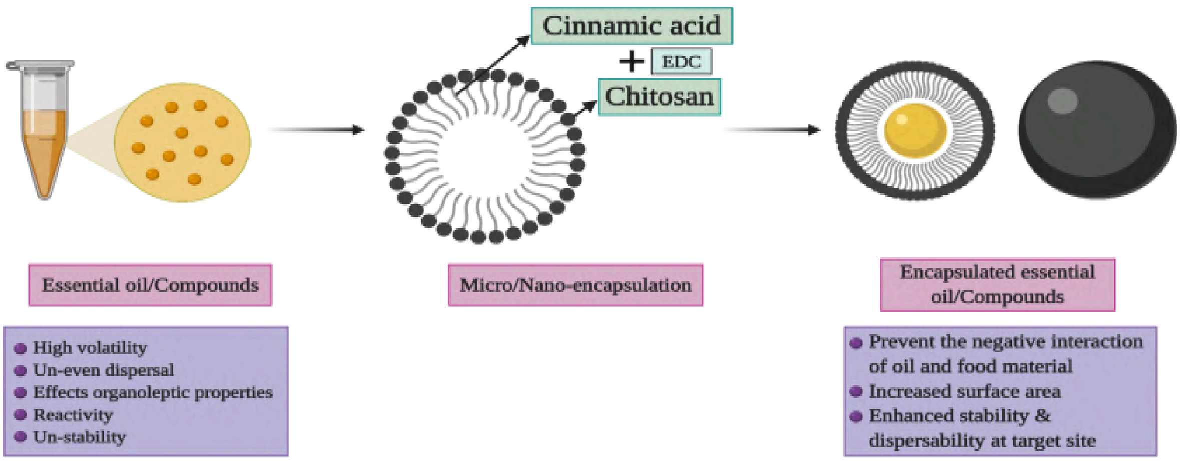


Fig.3 GCMS analysis of Mace essential oil

3.3. Synthesis and characterization of Ne-MEO



The packing process of free essential oil isolated from the mace was prepared using chitosan- cinnamic acid and EDC. EDC know as a zero-length cross linker and promote the nanogel formation. EDC dependent linkage of cinnamic acid to chitosan in liquid medium (water) forms nanomicelle that leads to self-organization and formation of hydrophobic cage or cavity. The formed hydrophobic cage or cavity have capacity to entrap the isolated mace essential oil and help in effective and controlled release in food system (Zhaveha *et al*., 2015)

Fig. 4 Extraction of Mace Essential oil using Clevenger hydrodistillation Method

3.4 Scanning electron microscope (SEM) images

Magnification in two dimensions is done using Scanning electron microscope (SEM). Obtained SEM photographs shows the prepared nanoparticles loaded with MEO have spherical shape with an average size of less than 100 nm. It was found that nanoscale ranges increase surface to volume ratio and improve the bioavailability of green chemicals present in MEO.

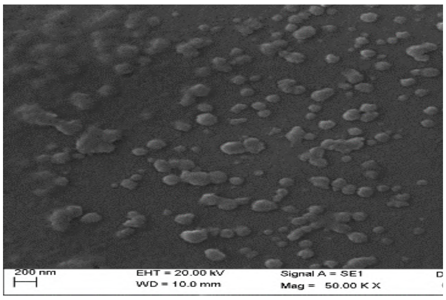


Fig.5 SEM image

3.5. Antimicrobial activity of nanoencapsulated mace essential oil

In vitro antifungal and antiaflatoxigenic efficacy of nanoencapsulated mace essential oil was checked against the A. *flavus and* other food borne mold and it was found that, nanoencapsulated mace essential oil causes complete inhibition of growth and Aflatoxin B1(AFB1) production at 1.7μL/mL, which was little lower than the free mace essential oil 2.0 μL/mL, it was also found that inhibitory efficacy Inhibition was directly proportional to the concentration of nanoencapsulated mace essential oil.

3.6. Antioxidant activity of mace essential oil

Further, mace essential oil and nanoencapsulated mace essential oil possess antioxidant activity or free radical scavenging activity. The assessment of the antioxidant activity was done using DPPH assay (Pukalskas, van Beek, & de Waard, 2005). Graphs, distinctly shows that free and encapsulated essential oil having strong free radical scavenging activity in concentration dependent manner (Khairan 2024).

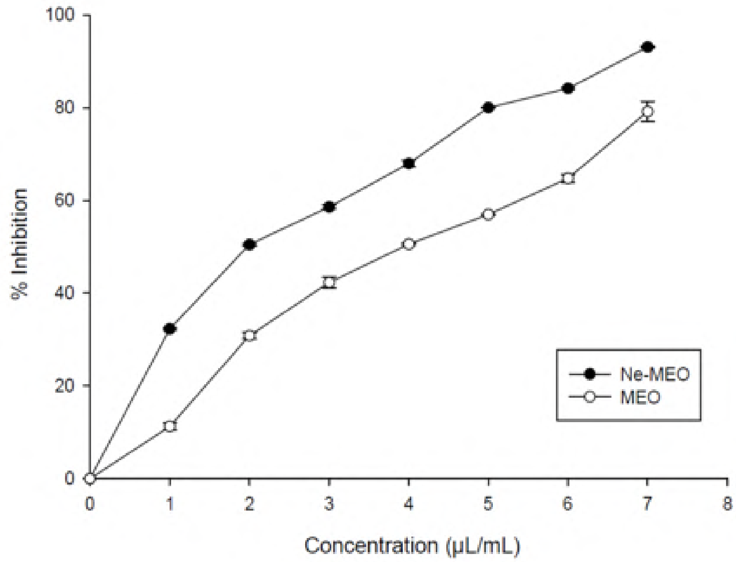


Fig7. Antioxidant activity of both free and nanoencapsulated mace essential oil

3.7 Defence mechanism of nanoencapsulated mace essential oil

Plant derived mace essential oil and after chitosan-based encapsulation might be target the pathogen plasma membrane which was ergosterol rich and ergosterol is one of the primary sterol present in cell membrane of target pathogen of A. *flavus* and help in maintaining the function and integrity of cell. Disturbance in ergosterol content disturbs homeostasis of cell and promote the leakage of major cellular ion results in fungal cell death (Kujur *et al.*,2017, Yadav *et al.*,2023). It also shows that that promising decrease of percentage ergosterol present in cell plasma membrane with increasing the dose mace essential. The ergosterol content decreased by 27%, 33% ,69% and 100%, respectively.

4. Conclusion

In conclusion this study suggests that the nonencapsulated mace essential oil is a promising natural food preservative as a green plant-derived against various food borne fungus and its toxin. The nonencapsulated mace essential oil are able to completely suppress the growth and toxin production at low concentration and are non-toxic to human and the environment.

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2. Understanding the Obesity–Hypertension Nexus: Mechanisms and Management Approaches

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Abstract

Two of the most common non-communicable diseases (NCDs) in the world are obesity and hypertension, and their combination is an important problem for public health. Obesity (BMI $\geq$ 25 kg/m^2) has reached epidemic levels, with nearly half the world's population expected to be obese by 2030. It is now accepted as a chronic, relapsing, and multifactorial condition that disrupts adipose tissue function and leads to adverse metabolic effects. Hypertension (HTN), describes as blood pressure 140/90 mmHg or higher, is one of the main modifiable risk factors for chronic renal disease, cardiovascular disease and stroke. Obesity and hypertension have strong reciprocal relationships. Through renin-angiotensin-aldosterone system dysregulation, insulin resistance, sympathetic nervous system activity initiation, decreased nitric oxide bioavailability, and pro-inflammatory adipokines, process obesity raises blood pressure. Globally, excess body weight accounts for a significant proportion of hypertension cases and obese individuals nearly three times more prone to be hypertensive than normal weight individuals. Generalised and central obesity both increases hypertension risk, with abdominal obesity independently raises systolic blood pressure. Men tend to develop obesity-related hypertension earlier, while postmenopausal women face higher prevalence due to hormonal changes. Management requires lifestyle changes, pharmacotherapy, and bariatric surgery for severe cases. However, population-level prevention remains the most cost-effective strategy. Integrated, precision-based interventions tailored to diverse populations are essential to reduce morbidity, mortality, and healthcare costs.

Key words: *Obesity, hypertension, blood pressure, BMI, non-communicable diseases,*

Introduction

Two of the most important non-communicable diseases (NCDs) causing problems for world health in the twenty-first century are obesity and hypertension (HTN). Obesity is defined as an abnormal or excessive accumulation of body fat that affects health. BMI >25 kg/m^2 (BMI-Body mass index) used for the evaluation of obesity for Asian Indian population. About half of the world's population would be obese by 2030, if prevalence continued on its current trend of acceleration (Venkatrao M *et al*., 2020). Definition of obesity according to Obesity Medicine Association is explain as, *"A chronic, relapsing, multifactorial, neurobehavioral disease, wherein an increase in body fat promotes adipose tissue dysfunction and abnormal fat mass physical forces, resulting in adverse metabolic, biochemical, and psychological health consequences,"* (Bays H E *et al*., 2021). Consistently high systolic blood pressure of 140 mmHg and/or diastolic blood pressure of 90 mmHg define as hypertension (HTN). Hypertension was recorded as one of the crucial risk factors for cardiovascular illnesses, stroke, and chronic renal disease, (HTN) (Mills *et al*., 2020). Because of their connections, these disorders exacerbate metabolic and cardiovascular risk in patients. Renal damage insulin resistance and systemic inflammation, are some of the ways that obesity causes hypertension. In result, obesity-related hypertension exacerbates cardiovascular outcomes, resulting in early morbidity and death. In low- and middle-income nations like India and worldwide, obesity and HTN have become more common in recent decades. Sedentary lifestyles, rapid urbanization, and dietary changes are accelerating factors for the higher prevalence of obesity and hypertension. This double burden has placed immense pressure on healthcare systems. Hence, for clinicians, researchers, and policymakers it is necessary to understand the association between obesity and hypertension in designing preventive and therapeutic interventions.

1. Pathophysiological relation between Obesity and Hypertension

The pathophysiological connection between obesity and hypertension involves a complicated relationship between the relationship of neuro-hormonal, renal, and vascular systems. Excess body fat, especially visceral body fat, induces overactivity of the sympathetic nervous system (SNS). Due to peripheral vasoconstriction blood pressure rises and elevated heart rate indued by this mechanism enhanced SNS activity. Furthermore, obesity is also linked with renin-angiotensin-aldosterone system (RAAS) activation, and this relationship elevates vascular resistance, salt retention and causes hypertension. In addition, surrounding fat deposition makes physical compression on the kidneys due to impaired renal function and sodium reabsorption. This impaired mechanism causes kidney volume overloaded which raises blood pressure. Additionally, pro-inflammatory cytokines and adipokines (like leptin) secreted by adipose tissue can alter vascular tone and baroreceptor sensitivity. These inflammatory mediators decrease the availability of nitric oxide and inhibit vasodilation by causing endothelial dysfunction and oxidative stress. Overall, these processes provide a strong pathophysiological basis that connects fat and hypertension without the need for insulin resistance (Parvanova, A., et a.,2024, Meouchy El. *et al*., 2022).

Obesity and hypertension have multifaceted relationship, involving complex interactions between metabolic, hormonal, renal, and vascular systems. Following several mechanisms explain how excess adiposity elevates blood pressure:

a. *Neurohormonal Activation*

- **Renin-Angiotensin-Aldosterone System (RAAS:** adipocytes produce angiotensinogen, which elevates levels of angiotensin II. Aldosterone encourages water and salt retention, which raises blood pressure, while angiotensin II is a strong vasoconstrictor that increases systemic vascular resistance (Engeli *et al*., 2003).
- **Sympathetic Nervous System overactivity:** Too much leptin secreted from adipose tissue triggers sympathetic activation, especially in the kidneys. Overactivity of sympathetic nervous system is also stimulated by leptin and hyperinsulinemia. This raises heart rate, salt retention, and renin release (Rahmouni K *et al*., 2010, Reaven G M *et al*., 2011, Ouchi *et al*., 2011).

b. *Insulin Resistance and Hyperinsulinemia*

Obesity, especially visceral fat (central obesity), induced insulin resistance. Hyperinsulinemia promotes sodium reabsorption in the kidneys. This mechanism impairs endothelial nitric oxide (NO) production, reducing vasodilation (Reaven G M *et al*., 2011).

c. ***Adipokines and Inflammatory Cytokines***

Various adipokine is secreted by metabolically active adipose tissue such as leptin. In obesity, leptin resistance suppresses appetite control which shows hypertensive effect. Adiponectin is normally vasoprotective and anti-inflammatory, but reduced in obesity, contributing to vascular dysfunction. Pro-inflammatory cytokines (TNF-α, IL-6, CRP) promotes endothelial dysfunction, vascular remodelling and oxidative stress (Ouchi *et al*., 2011).

d. ***Renal and Mechanical Factors***

Visceral fat accumulation increases intra-abdominal pressure. This compresses the kidneys and impairs natriuresis (Hall *et al*., 2015). This results in sodium and fluid retention, and prolonged hypertensive condition.

e. ***Vascular Remodelling and Endothelial Dysfunction***

Excess free fatty acids and oxidative stress damage the endothelium, reduce nitric oxide bioavailability, and increase arterial stiffness. These vascular changes raise systemic resistance and induce hypertension. Based on above mechanisms it is observed that obesity acts as risk factor not only for hypertension, but it also acts as driver of its progression and related complications.

Mechanism	**Effect on BP**	**References**
Renin-Angiotensin-Aldosterone System (RAAS) activation	Adipocytes increase angiotensinogen: Increased Ang II/aldosterone leads to vasoconstriction & sodium retention	Engeli *et al*., 2003; Hall *et al*., 2015
Sympathetic nervous system overactivity	Leptin/insulin resistance stimulate SNS: Elevated heart rate, renin release, renal sodium reabsorption	Rahmouni K., 2010; Hall *et al*., 2015
Adipokines and inflammation (TNF-α, IL-6, decreased adiponectin)	Endothelial dysfunction, oxidative stress, vascular remodelling	Ouchi *et al*., 2011
Insulin resistance and hyperinsulinemia	Renal sodium retention, reduced NO bioavailability, vascular stiffness	Reaven G M *et al*., 2011

Renal compression and impaired natriuresis	Visceral fat raises intra-abdominal pressure: leads to kidney compression leads to sodium/fluid retention	Hall *et al.*, 2015
Endothelial dysfunction and arterial stiffness	Decreased Nitric oxide, increased oxidative stress leads to elevated systemic vascular resistance	Hall *et al.*, 2015

Table 1: Mechanisms linking obesity to hypertension.

Epidemiological Evidence

Epidemiological data strongly support the strong relationship between obesity and hypertension among populations. Excess body weight was found to be responsible for 28% of hypertension in women and 26% in men (Wilson *et al.*, 2002). Risk of hypertension was almost three times higher for obese people than for people of normal weight.

A follow-up study revealed a clear positive correlation between weight increase, BMI, and the risk of hypertension in over 80,000 women (Huang *et al.*, 1998).

1. Indian Context

Obesity is seen as one of the most important modifiable risk factors for hypertension because excess body fat, especially abdominal fat. Obesity, both central and general, dramatically increases the risk of high blood pressure, which speeds up metabolic and cardiovascular problems. Using nationally representative data from NFHS-5 (2019-21), Gupta *et al*. (2025) showed that people with both abdominal obesity (based on waist-hip ratio criteria) and general obesity (high BMI) had significantly higher odds of hypertension. Roughly twice times for women and thrice times for men in contrast to normal individuals (Gupta *et al*., 2025).

Furthermore, in the absence of genialised obesity, only abdominal obesity increases systolic and diastolic blood pressure roughly 3-5 mmHg. independent of generalised obesity (BMI). Notably, data emphasized that abdominal obesity may be more powerful and independent predictor of hypertension in the Indian context than BMI alone (Gupta R D., *et al*., 2025). This pattern is also supported by several other Indian studies. Abdominal obesity assessing anthropometric parameters such as waist-hip ratio (WHR), waist circumference (WC), and waist-to-height ratio (WHtR), were better predictors of, cerebrovascular and coronary artery disease than BMI among hypertensive patients (Gupta P *et al*., 2024).

Gender and age are important factors in the development of hypertension associated with obesity. According to research, males were more likely than women to suffer from cardiovascular problems, such as obesity-related hypertension, almost ten years earlier, highlighting their earlier exposure to risk (WHO, 2023). On the other hand, South Asian data indicate that women bear greater burden as they aged. For example, women between the ages of 65-74 years have 5.3% higher prevalence of hypertension than men in the same group, and this gap increases to 11.8% for those 75 years and older (Ramezani T *et al*., 2022). These trends highlight how crucial it is to modify preventative tactics according to age and gender. Women are more susceptible to obesity-related hypertension after menopause, primarily because of hormonal changes, whereas men are more likely to develop this condition earlier in life. When combined, the data highlight obesity as a significant modifiable cause of hypertension in a variety of age categories and demographics.

Clinical Manifestations and Complications

Hypertension rarely occurs alone in obese individuals; instead, it is frequently associated by a variety of other cardiometabolic illnesses. Compared with their lean counterparts, obese people tend to develop elevated blood pressure earlier, often in their middle age (30-40 years). In obese individuals' hypertension was strongly linked with cardiovascular complications such as heart failure, atrial fibrillation and ischemic heart disease. Obesity-related glomerulopathy further contributes to the occurrence of proteinuria and chronic kidney disease (CKD), a process accelerated by coexisting hypertension (Kambham *et al*., 2001). In addition, obesity related obstructive sleep apnea (OSA), independently increases the risk of hypertension through intermittent hypoxia (Somers *et al*., 2008). Collectively, these interlinked mechanisms underscore that hypertension in obesity is rarely isolated but part of a complex cardiometabolic syndrome that accelerates cardiovascular and renal complications.

2. Assessment and Diagnosis

a. *Anthropometric Measurements*

BMI>25.0 kg/m^2 is used to assess generalised obesity (Table 2), whereas, for central obesity (abdominal obesity) assessment, waist circumference (WC) (Table 3) and, hip circumference (HC), waist-to- hip ratio (WHR) anthropometric parameters are used. These abdominal obesity assessment anthropometric parameters are more predictive about hypertension and

BMI (kg/ m^2)	Classification
<18.5	Underweight
18.5-22.9	Normal (not obese)
23.0-24.9	Overweight
>25.0	Obese

Table 2: Anthropometric parameter criteria of generalised obesity for Asian Indians (Venkatrao, M., *et al*., 2020).

Gender	WC Cut-off (cm)
Men	>90 cm
Women	>80 cm

Table 3: WHO Criteria of central obesity for male and female.

b. *Blood Pressure Assessment*

The Eighth Joint National Committee (JNC-8) states that when a person's diastolic blood pressure increases 90 mmHg or higher, or when their systolic blood pressure increases 150 mmHg or higher, pharmacologic treatment should start in the population of people 60 and older. Patients should aim for systolic blood pressure (SBP) <150 mmHg and diastolic blood pressures (DBP) <90 mmHg. If the systolic blood pressure drops below 140 mmHg, therapy does not need to be altered as long as there are no negative effects on health. At least two visits are advised for the confirmation of HTN. In the normal population under 60, medication should start when the diastolic blood pressure reaches 90 mmHg or higher, and/or when the systolic blood pressure reaches 140 mmHg or higher. Targeted SBP and DBP is <140 mmHg and <90 mmHg respectively in the population under medicinal treatment.

c. *Laboratory Investigations*

Blood (biochemical) markers are used to investigate the risk of hypertension which provide a comprehensive view of patients metabolic and cardiovascular risk status.

The lipid profile usually shows reduced high-density lipoprotein cholesterol (HDL-C), whereas increased triglyceride and low-density lipoprotein cholesterol (LDL-C) is observed. In addition to insulin resistance (measured by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)), blood glucose markers (glycemic markers) including fasting blood glucose (FBP) and glycated-hemoglobin (HbA1c) are often increased, which suggest poor glucose metabolism. Obesity related inflammatory markers, such as interleukin-6 (IL-6), uric acid, and high-sensitivity C-reactive protein (hs-CRP) are seen increased, all these causes chronic low-grade inflammation. Early signs of nephropathy include elevated blood creatinine, decreased estimated glomerular filtration rate (eGFR), and microalbuminuria, which indicate that kidney function may already be impaired. When taken as a whole, these indicators demonstrate the pervasiveness of obesity and its critical involvement in the emergence of hypertension and associated problems.

Management Approaches

A comprehensive strategy that targets both the simultaneous control of excess body weight and blood pressure lowering is necessary for the effective management of obesity associated hypertension.

Lifestyle Interventions

An essential component of both the prevention and treatment of hypertension is dietary management. The Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) diet are two dietary patterns that have been repeatedly backed by research. The DASH diet limits sodium, saturated fats, and added sugars while promoting a greater consumption of fruits, vegetables, whole grains, and low-fat dairy products. According to research, following this routine can dramatically lower blood pressure, and changes are sometimes seen in as little as a few weeks (Singh *et al.*, 2023). Simultaneously, the Mediterranean diet, which is based on the historic eating patterns of people in the Mediterranean region, emphasizes whole grains, legumes, nuts, fish, and olive oil as staple foods. It also calls for moderate dairy consumption and a limited intake of red meat and sweets. Its abundance of omega-3 fatty acids and antioxidants helps to protect the heart and improve blood pressure control (Critselis *et al*., 2020). The Mediterranean and DASH diets restrict consumption of foods high in saturated fat, refined carbohydrates, and sugar-sweetened beverages, all of which lead to vascular and metabolic dysfunction. Because of its complimentary advantages, a blended model often referred to as the Med-DASH diet has drawn attention. It has been shown to be more successful in reducing cardiovascular risk and improving overall metabolic health (Filippou *et al*., 2024).

Both medication and lifestyle interventions are essential for controlling hypertension, especially in obese people. Evidence supports at least 150 minutes per week of moderate-intensity aerobic exercise in addition to resistance training, demonstrating the need of regular physical activity. These practices have been demonstrated to decrease visceral fat, enhance endothelial function, and lower blood pressure (Monfared *et al*., 2024). Maintaining a healthy weight is also important; a 5–10% reduction in body weight can lower systolic blood pressure by 5-20 mmHg. Compared to short-term dietary changes, long-term adherence to lifestyle changes typically yields stronger long-lasting effects (Hiremath *et al*., 2025). Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), which lower blood pressure while protecting the kidneys, are frequently the first pharmacological treatments for hypertension. While beta-blockers are typically saved for individuals with particular diseases, like ischemic heart disease, calcium channel blockers and thiazide diuretics are commonly used as supplemental therapy (Noone *et al*., 2020). By encouraging substantial weight loss, enhancing blood sugar regulation, and reducing blood pressure, drugs such as GLP-1 receptor agonists have demonstrated promise for people who are unable to lose enough weight with lifestyle changes alone. Orlistat is an additional choice; it inhibits the absorption of fat and has been linked to mild drops in blood pressure (Hiremath *et al*., 2025). Bariatric surgery has been demonstrated to result in significant and sustained weight loss among surgical procedures, along with notable enhancements in blood pressure, metabolic syndrome, and cardiovascular health in general (Schauer *et al*., 2017).

a. Preventive Strategies

Prevention is widely recognized as a more cost-effective strategy than treatment when addressing the dual burden of obesity and hypertension. At the individual level, lifestyle counselling, routine screening, and early intervention in obese individuals considerably decrease the risk of progression to chronic disease. These approaches not only enhance health outcomes but also reduce long-term healthcare expenditures (Hall *et al*., 2021). Community-level interventions, such as health promotion campaigns that encourage active living, balanced nutrition, and awareness about excessive salt and sugar intake, have proven effective in shifting population behaviours and reducing cardiovascular risk (Chandra, 2024). Policy-level strategies +including taxation of sugar-sweetened beverages, mandatory front-of-pack food labelling, regulation of junk food advertising, and urban planning that promotes physical activity are essential tools in shaping healthier environments and reducing obesogenic exposures (American Psychological Association, 2018). Additionally, workplace wellness programs that incorporate physical activity breaks, healthier cafeteria options, and stress reduction initiatives have shown promise in improving employee health and productivity while lowering healthcare costs (Hall *et al*., 2021). Together, these multilevel prevention strategies form a comprehensive framework for mitigating the increasing public health crisis posed by obesity and hypertension.

Conclusion

The relationship between obesity and hypertension is well established and clinically significant. Obesity contributes to hypertension through neurohormonal, inflammatory, renal, and vascular mechanisms, while epidemiological evidence confirms its role as a crucial modifiable risk factor. Clinically, obesity-related hypertension is related with increased cardiovascular and metabolic risk, necessitating comprehensive assessment and early intervention. GLP-1 receptor agonists and dual incretin therapies (GLP-1/GIP co-agonists) are examples of novel agents that have demonstrated notable effectiveness in lowering blood pressure, improving glycemic control, and reducing weight. As such, they are promising options for treating obesity-related hypertension with drugs. These substances work by altering neurohormonal and metabolic pathways connected to blood pressure regulation, among other mechanisms beyond hunger suppression. Their incorporation into clinical practice may cause management approaches to change in favour of more individualized, mechanism-based treatments. Management requires a multidisciplinary approach combining lifestyle modification, pharmacotherapy, and surgical interventions where necessary. Preventive measures at the individual, community, and policy levels are essential to curb the epidemic.

Future directions: To lessen the burden of obesity-related hypertension, future studies should concentrate on precision medicine, innovative treatments, and population-specific approaches. Combining clinical, lifestyle, and pharmaceutical approaches can greatly improve the long-term prognosis for those who are impacted.

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3. Bovine coccidiosis in a female Holstein Friesian calf: A Case Report

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Abstract

Bovine coccidiosis is an economically important protozoan disease caused by various species of *Eimeria.* It affects the calves, leading to significant losses in livestock productivity. The present study reports a postmortem case in a 45-day-old female Holstein Friesian calf with a history of persistent diarrhea unresponsive to treatment. Clinical examination revealed pale mucous membranes and rigor mortis in hindlimbs. Necropsy findings revealed enlarged mesenteric lymph nodes, diffusely corrugated and thickened intestinal mucosa, pulmonary emphysema, and a fibrinous layer on the liver. Impression smears from the intestine stained with the Ziehl-Nelsen acid-fast method revealed coccidial oocysts. Histopathological examination (H&E stain) showed coccidial sporozoites and merozoites in the lamina propria, severe villous sloughing, multifocal congestion, and mixed inflammatory cell infiltration. This case highlights coccidiosis as a persistent challenge in calf rearing, often exacerbated by environmental stressors such as poor hygiene and overcrowding. Emerging drug resistance in *Eimeria spp.* underscores the need for innovative control strategies integrating early diagnosis, targeted therapeutics, and farm-level biosecurity measures.

Key words: *Coccidiosis, calf, Eimeria, pathology, sustainable livestock health*

Introduction

Bovine coccidiosis is a protozoan disease caused by various species of *Eimeria* affecting the gastrointestinal tract of cattle (Hastutiek *et al*., 2025). Coccidiosis has been indicated as an important cause of diarrhea in calves (Radostits *et al*., 1994). This disease is an economic important disease as the protozoan damage the lining epithelium of the intestine leading to anorexia, emaciation, impaired feed conversion diarrhoea, dysentery, unthriftiness, anemia and increased vulnerability to other diseases (Bohrmann, 1991). Long-term consequences of *Eimeria* infections include decreased performance in heifers and cows (Lassen & Ostergaard, 2012).

Material and method

A 45-day-old female Holstein Friesian calf was submitted for necropsy examination to the Department of Veterinary Pathology, GADVASU, Ludhiana, with a clinical history of persistent diarrhea unresponsive to therapeutic management.

A detailed necropsy examination was performed on the carcass to record the gross pathological changes. Impression smears were prepared from intestinal scrapings, which were examined directly under the microscope. In addition, smears were subjected to Ziehl–Neelsen (ZN) staining for detection of acid-fast organisms. Representative tissue samples were collected in 10% Neutral Buffered Formalin (NBF) for further histopathological examination. The formalin-fixed tissues were processed by routine paraffin embedding and microtome cutting (4 µm). The sections were stained with routine Hematoxylin and Eosin (H&E) stains (Luna, 1968). Microscopic lesions were examined and photographed.

Results

Gross Findings

On external examination, the calf exhibited pale mucous membranes along with rigor mortis in the hindlimbs Detailed necropsy findings revealed significant pathological alterations, including markedly enlarged mesenteric lymph nodes (Figure 1), diffusely corrugated and thickened intestinal mucosa (Figure 2) and multifocal ecchymotic hemorrhage suggestive of severe enteric involvement, and the presence of a fibrinous layer deposited on the liver surface, which was indicative of fibrinous perihepatitis.

Impression smear examination

Impression smears prepared from the intestinal contents, when observed under a direct light microscope, revealed the presence of coccidial oocysts (Figure 3), and differential diagnosis from Johne's disease was achieved by Ziehl–Neelsen acid-fast staining, which demonstrated only acid-fast coccidial oocysts (Figure 4), thereby establishing the role of coccidial infection in the pathogenesis of the reported case.

Histopathological Findings

In the small intestine, multifocal hemorrhages were observed in the lamina propria accompanied by sloughing of the intestinal villi, indicative of extensive mucosal damage. The epithelial layer harbored various developmental stages of coccidial schizonts (Figure 5), reflecting active parasitic replication within the enterocytes. Significant infiltration of polymorphonuclear cells was noted throughout the mucosal layer (Figure 6), suggesting a strong acute inflammatory response to the infection. Examination of lymphoid organs revealed lymphoid depletion in the spleen, pointing toward immunosuppression or stress-associated lymphoid involution. The liver exhibited mild degenerative changes, with the deposition of a fibrinous layer on its outermost surface, consistent with early fibrinous perihepatitis. Collectively, these histopathological findings corroborate the severe systemic impact of coccidial infection in this young calf, affecting both the gastrointestinal tract and multiple organs.Thus, based on the comprehensive evaluation of gross lesions, histopathological alterations, and confirmatory staining results, a definitive diagnosis of coccidiosis by *Eimeria* sp. was established.

Discussion

The life cycle of *Eimeria* starts with the ingestion of the unsporulated oocysts in the environment by animals which sporulates in the intestine under favourable conditions. Sporulation leads to release of sporocysts which contain sporozoites. The sporozoites then invade the epithelial cells of the intestine and undergo schizogony leading to formation of multinucleated schizont containing numerous merozoites. After Schizogony, sexual replication (gametogony) occur leading to formation of microgametes (male gametes) and macrogametes (female gametes). Then zygote is formed through fusion of these microgametes and macrogametes and this zygote develop into an oocyst. The oocysts are shed in the environment and the cycle repeats (Sarfaraz *et al*., 2025). In the livestock industry, particularly in cattle, *Eimeria* spp. caused serious infections and had a significant effect on the economy through weight loss, mortality and cost of treatment (Lassen & Ostergaard, 2012).

Figure 1: Enlarged Mesenteric Lymph Node

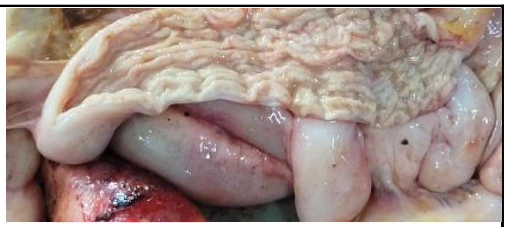

Figure 2: Highly corrugated and thickened lumen of whole small intestine

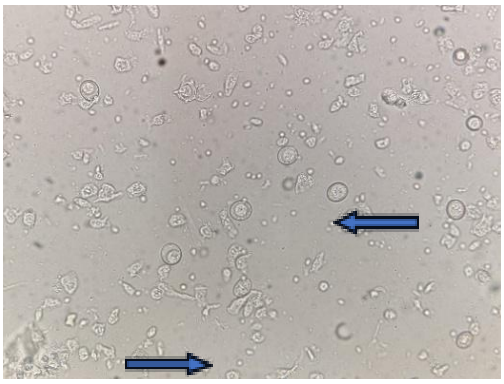

Figure 3: Impression smears from intestinal scraping reveals coccidial oocyst (blue arrow) under direct microscopic examination

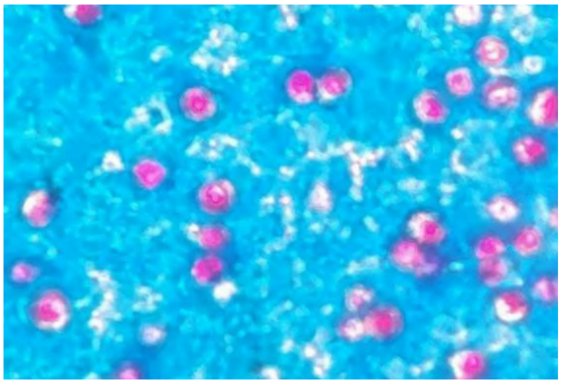

Figure 4: ZN staining of impression smear reveals coccidial oocyst

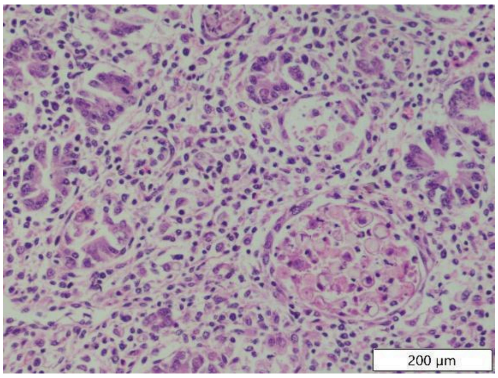

Figure 5: Various stages of Coccidial schizonts in the epithelium of small intestine was observed under high power (H&E, 40X)

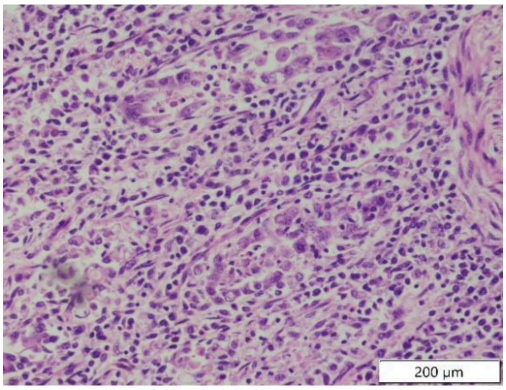

Figure 6: Infiltration of polymorphonuclear cells in the mucosal layer of intestine was observed under high power (H&E, 40X)

Conclusion

This case highlights coccidiosis as a persistent challenge in calf rearing, often exacerbated by environmental stressors such as poor hygiene and overcrowding. Emerging drug resistance in *Eimeria spp.* underscores the need for innovative control strategies integrating early diagnosis, targeted therapeutics, and farm-level biosecurity measures. Future research should focus on sustainable alternatives such as vaccines and integrated parasite control to reduce reliance on anticoccidial drugs.

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4. Therapeutic Nutrition in Non-Alcoholic Fatty Liver Disease (NAFLD): Evidence-Based Dietary Strategies

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Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is largely shaped by lifestyle and dietary habits, making nutrition a central factor in both prevention and treatment. Dietary strategies emphasize balancing macronutrients, meeting micronutrient requirements, and encouraging healthier eating practices. Consuming diets high in complex carbohydrates, fibre, unsaturated fats, lean proteins, and antioxidant-rich foods has been shown to decrease liver fat accumulation and enhance insulin sensitivity. In contrast, frequent intake of saturated fats, refined sugars, and processed foods accelerates disease progression. Research highlights the effectiveness of nutrient-dense dietary patterns—particularly the Mediterranean diet and calorie restriction—in slowing NAFLD progression, improving liver function, and reducing related metabolic risks.

Definition and Epidemiology - Non-alcoholic fatty liver disease (NAFLD), first described by Schaffner in 1986, is defined as a clinical condition marked by excessive fat accumulation (steatosis) in more than 5% of hepatocytes, occurring in individuals with minimal or no alcohol intake (<20 g/day in women and <30 g/day in men) **[1]**. NAFLD is regarded as the hepatic manifestation of metabolic syndrome and has emerged as a significant public health concern owing to its high prevalence. It comprises two distinct clinicopathological entities: simple steatosis, which generally follows a stable, non-progressive course, and non-alcoholic steatohepatitis (NASH), a more severe form that may progress to cirrhosis and hepatocellular carcinoma **[2]**. NASH, first described in 1980, is defined by persistent liver inflammation. Currently, it is recognized as a highly heterogeneous condition, primarily differentiated by the presence or absence of fibrosis

[1]. Approximately 80–90% of NAFLD cases present as simple steatosis, which is typically benign and non-progressive, while the remaining 10–20% correspond to

NASH, a more severe phenotype that carries the risk of progression to cirrhosis or hepatocellular carcinoma (HCC) **[3]**. NAFLD frequently coexists with type 2 diabetes and obesity, and their combined effects intensify the risk of developing more severe liver disease. Studies report that NAFLD affects nearly 75% of individuals with type 2 diabetes and about 90% of those with obesity, highlighting its strong link with both conditions. Furthermore, NAFLD contributes to the increasing prevalence of type 2 diabetes and its complications, while diabetes, in turn, accelerates the progression of NAFLD to advanced stages, including NASH, fibrosis, and hepatocellular carcinoma (HCC) **[4]**.

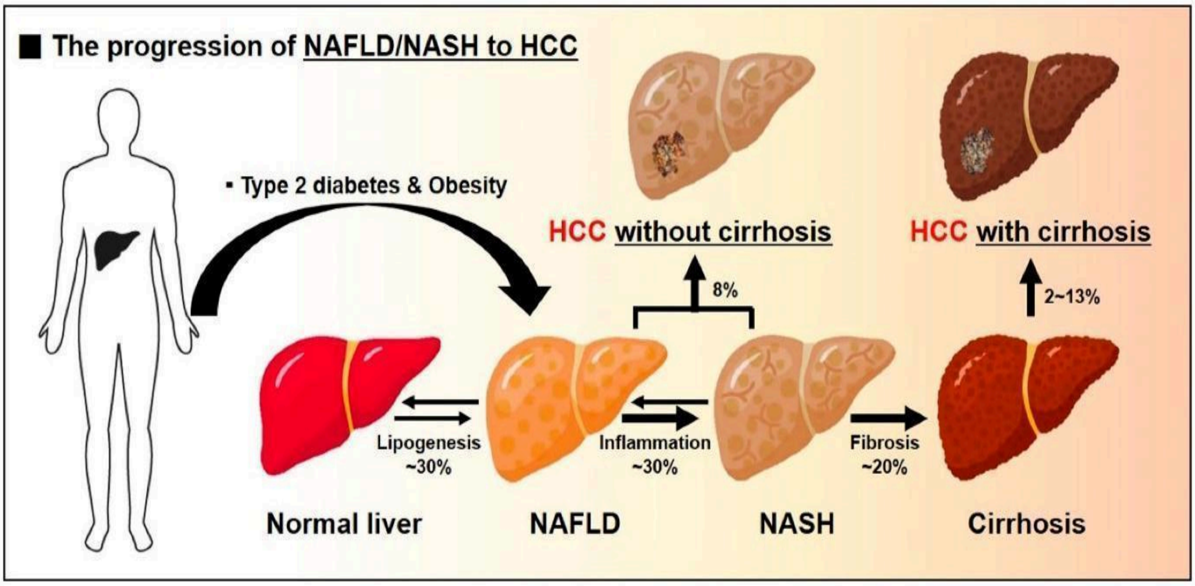

Figure-1: The presence of type 2 diabetes and obesity accelerates the transition of NAFLD and NASH to advanced stages, including hepatocellular carcinoma (HCC).

NAFLD, now redefined as MAFLD (Metabolic dysfunction–Associated Fatty Liver Disease) - An International team of specialists began working toward a consensus to provide a better word for the illness in the beginning of 2020. The phrase "metabolic dysfunction-associated fatty liver disease" (MAFLD) was coined through a two-phase Delphi process. Two of the seven risk factors must be present for a diagnosis to be made. Risk variables include blood pressure, insulin resistance score based on the homeostasis model, prediabetes, plasma triglycerides, plasma HDL cholesterol, waist circumference, and plasma high sensitivity C-reactive protein. When one of these three metabolic risk variables is present along with hepatic steatosis, MAFLD is diagnosed **[5]**.

This chapter highlights how the metabolic causes of fatty liver disease are reflected in MAFLD, highlighting the critical role that nutrition plays in managing the condition. One main tactic to halt the advancement of the condition is dietary modification, which aims to reduce weight, enhance insulin sensitivity, and reduce hepatic fat. In order to prevent MAFLD and its associated problems, it is crucial to identify and promote healthy dietary patterns as modifiable objectives, as imbalanced nutrition is not only associated with NAFLD but also serves as a general risk factor **[6]**.

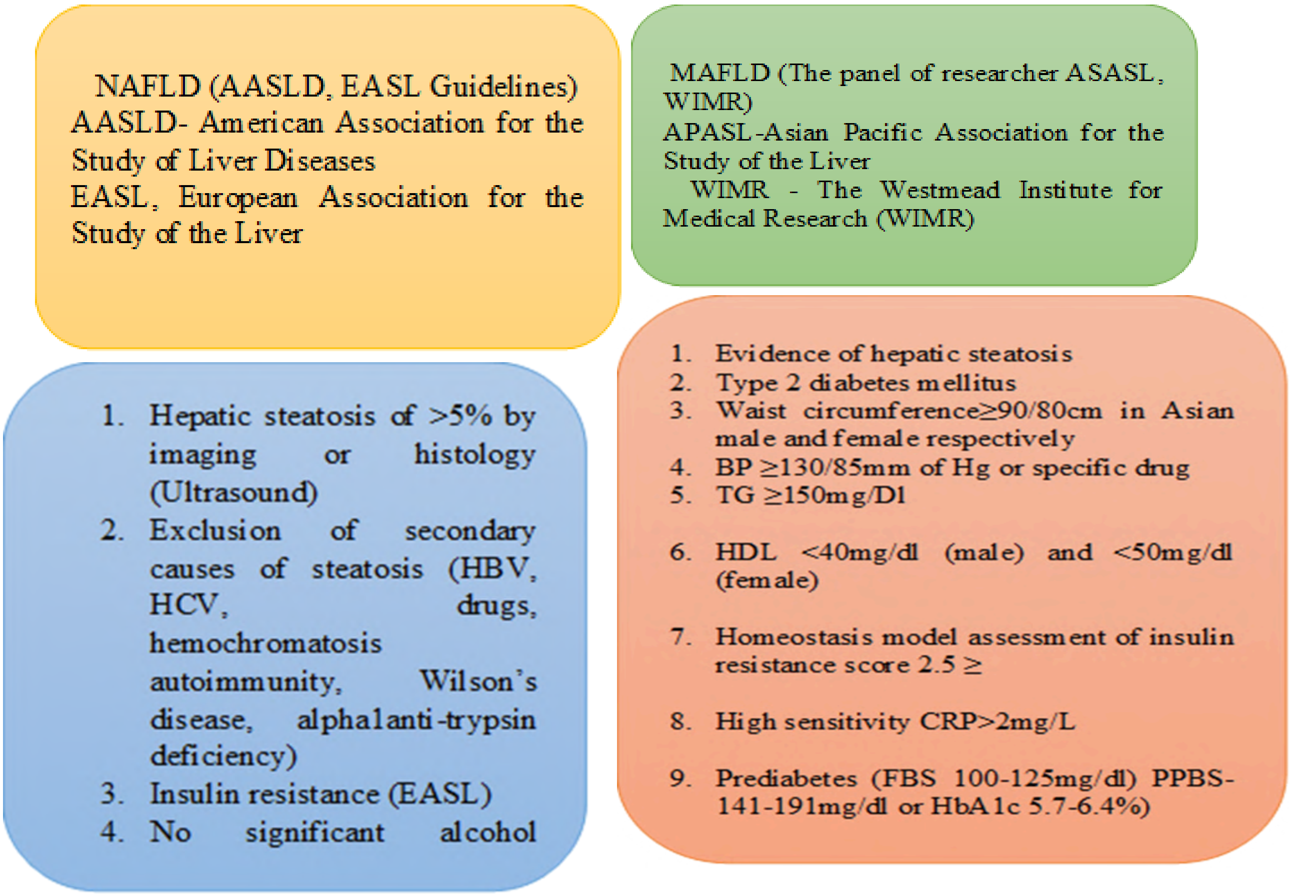

Figure-2: A comparative overview of diagnostic criteria for NAFLD and MAFLD [7].

Nutritional Approaches in the Management of NAFLD-

Dietary Factors and Mechanistic Pathways in NAFLD Progression - Diet plays a dual role in NAFLD, acting both as a contributing factor and a therapeutic tool. While the Western dietary pattern is linked to disease progression, the Mediterranean diet has been shown to improve histological features and support regression of liver damage. Overall, comprehensive dietary modifications appear more effective in managing NAFLD than changes in individual nutrient composition.**[8]** MAFLD exhibits sex-based differences that may be influenced by distinct dietary patterns across age groups, though the precise connection remains unclear. Overall, consumption of a hypercaloric diet—especially one high in trans fats, saturated fats, cholesterol, and fructose-sweetened beverages—promotes visceral fat accumulation, hepatic lipid deposition, and progression to steatohepatitis and fibrosis. Conversely, reducing intake of these foods and incorporating monounsaturated fatty acids (MUFAs) has shown both preventive and therapeutic benefits **[9]**.

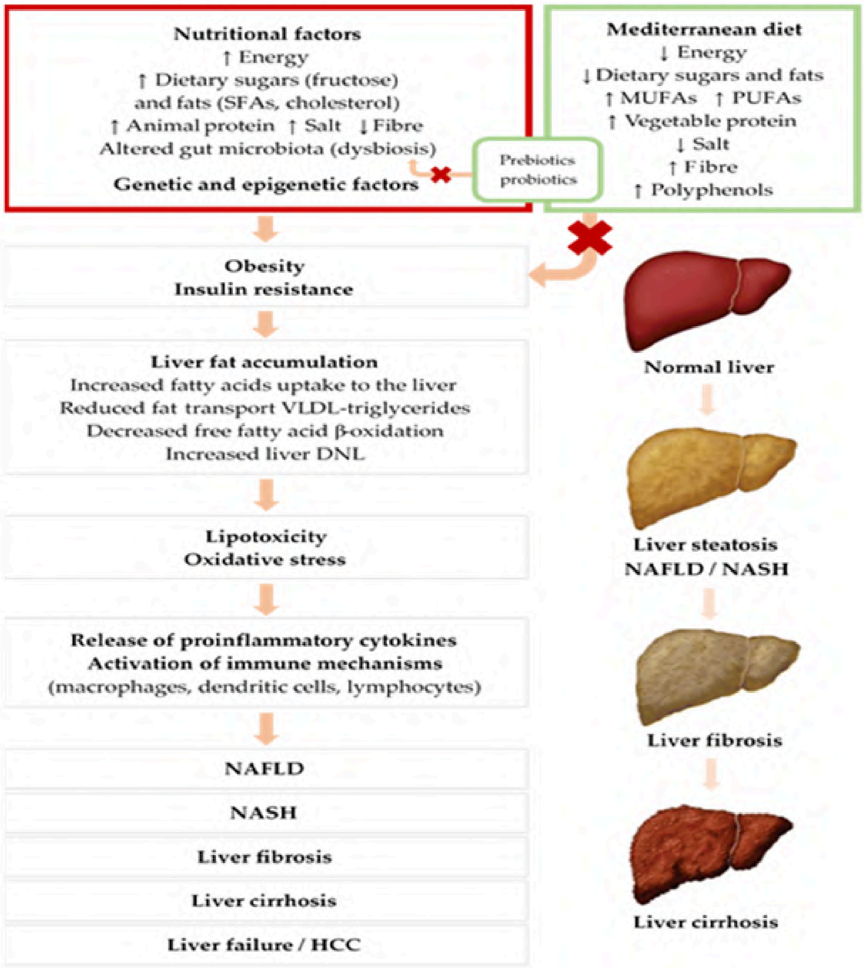

Figure 3- Mechanisms of NAFLD Development and Nutritional Influences

Upward arrows (↑) represent increased intake, while downward arrows (↓) represent decreased intake.

Abbreviations: SFAs – saturated fatty acids; MUFAs – monounsaturated fatty acids; PUFAs – polyunsaturated fatty acids; VLDL – very low-density lipoprotein; DNL – de novo lipogenesis **[10]**

Dietary Patterns in MAFLD-

- ➔ **Mediterranean Diet (MD)-** The Mediterranean diet is widely recognized for its metabolic benefits. Rich in fruits, vegetables, legumes, whole grains, nuts, olive oil, and fish, the MD improves insulin sensitivity, reduces liver fat, and lowers cardiovascular risk **[11]**.
- ➔ **DASH Diet -** In people with MAFLD, the Dietary Approaches to Stop Hypertension (DASH) diet, which emphasizes whole grains, lean protein, reduced salt intake, and an abundance of fruits and vegetables, has been demonstrated to improve metabolic risk indicators and liver steatosis **[12]**.
- ➔ **Low-Carbohydrate and Intermittent Fasting –** Although long-term commitment is difficult, low-carb diets can lower hepatic fat level more quickly than low-fat diets. Independent of weight loss, time-restricted eating and intermittent fasting have also demonstrated promise in lowering intrahepatic fat and enhancing metabolic indicators **[13]**.

Macronutrient Composition and Key Nutrients- The liver uses three main macronutrients—carbohydrates, fats, and proteins—for energy metabolism. It extracts almost two-thirds of the glucose and other monosaccharides absorbed from the gastrointestinal tract. Depending on the body's energy needs, glucose is either stored as glycogen or oxidized to produce energy. However, because hepatic glycogen storage is limited, excess carbohydrates are converted into triglycerides via de novo lipogenesis (DNL), a major mechanism in hepatic steatosis **[14]**.

- ➔ **Carbohydrates and Sugars-** Excessive intake of refined carbohydrates and fructose promotes hepatic lipogenesis and progression of MAFLD. Reducing sugar-sweetened beverages is a critical dietary strategy **[15]**.
- ➔ **Fats**
    - Saturated fats: Associated with worsening steatosis and metabolic dysfunction
    - Monounsaturated fats (MUFA) and Polyunsaturated fats (PUFA**)**: Particularly omega-3 PUFA, reduce liver fat and systemic inflammation **[16]**.

➔ **Proteins-** Protein-rich diets may enhance satiety and improve body composition, though the optimal quantity and source remain debated. Plant-based proteins may offer additional metabolic benefits **[17]**.

Table -1 Recommended Macronutrients [9]

Macronutrients	Mechanism (hepatic role)	Food source
Whole grains **[18]**	whole grains have the ability to alter the makeup of the human gut microbiota, including Lactobacillus and Bifidobacteria, due to phenolic acid have ferulic acid reduce inflammation, Betanin	Cereals
MUFAS and PUFAS **[19]**	It improves AST, ALT, and GGT levels in liver by activating the hepatic energy-sensing cascade and upregulating the peroxisome proliferator-activated receptor-α (PPAR-α), which lowers de novo fatty acid production and boosts fatty acid oxidation, hence lowering liver steatosis	Olive oil, advocates, nuts and nut oils, pumpkin seeds, and flaxseeds
Omega 3 **[20]**	Hepatocyte lipid metabolism is modulated by endogenous PPARα ligands. down-regulates pro-inflammatory genes, such as TNF-α and IL-6	Seafood, eggs, and meat, Fatty fish
Vegetable proteins **[21]**	By lowering sterol regulatory element-binding protein 1c levels, inhibiting lipogenesis, promoting lipolysis by activating peroxisome proliferator-activated receptors (PPARα), and lowering oxidative stress, anthocyanins may stop the progression of liver damage associated with non-alcoholic fatty liver disease (NAFLD), plant metabolites found in fruits that prevent hepatic fibrogenesis, decrease lipogenesis, and encourage hepatic lipolysis	Whole grains, cereals, seeds, nuts, legumes, vegetables, peas, and soybeans
Prebiotic, probiotic, fiber **[22]**	enhancing glucoregulation, decreasing body fat, and altering the gut microbiome.	garlic, asparagus, leeks, onions, cereals, yogurt

Table -2 Avoidable Macronutrients- [9]

Macronutrients	Mechanism	Food source
Simple carbohydrates **[23]**	result in significant elevations in liver fat	Fructose, refined carbohydrates (sucrose, honey, and syrup)
Saturated and animal proteins **[24]**	Two major risk factors for cardiometabolic disorders, including non-alcoholic fatty liver disease (NAFLD), are saturated fatty acids (SFA) and cholesterol, which are found in meat.	Red meat, butter, dairy products, vegetable oils (coconut, palm) processed foods (sausages, desserts)
Trans Fat **[25]**	Increased expression of genes linked to fibrosis, raised liver triglyceride and cholesterol levels, and enhanced ALT activity	Partially hydrogenated vegetable oil, desserts

Abbreviation- ALT-Alanine Amino transaminase, AST-Aspartate Amino transferase, GGT- Gama-Glutamyl transferase

Micronutrients composition- The World Health Organization defines micronutrients as nutrients needed in extremely small levels, usually in milligram or microgram amounts, to maintain essential physiological processes. They include minerals, vitamins, carotenoids, and electrolytes, all of which are necessary for intermediate metabolism, enzyme activity, and the body's metabolic reaction to disease. Minerals like calcium, phosphorus, and zinc, as well as electrolytes like sodium, chloride, and potassium, are inorganic substances that are essential for many enzyme functions, tissue construction, pH control, neural communication, and muscular contraction. Electrolytes and minerals frequently work together to supply electrical charge and sustain cellular activity **[26].**

Table-3 Role of various Micronutrients in NAFLD [9]

Micronutrients	Hepatic role	Food source
Vitamin E **[27]**	Antioxidant activity, which protects liver cells from damaged caused by oxidative stress and inflammation	Plant based oils- wheat germ oil and sunflower oil Nuts and seeds- almonds peanuts Fruits-Mangoes, kiwi, and avocados Vegetables- spinach, red capsicum
Vitamin D **[28]**	Plays an "on-off switch" role in liver fat. Activating fat burn gene and deactivating fat stored gene, regulates inflammation	Oily fish, red meat, egg yolks,
Vitamin C **[29]**	Vitamin C prevents fatty changes. Unexpectedly, the development of steatosis was prevented by vitamin C, a strong hydrosoluble antioxidant.	Citrus fruits, peppers, kiwi fruits, tomatoes, dark leafy greens, like broccoli and Brussles.
Vitamin A **[30]**	Vitamin A metabolites are important for adipose tissue development, maturation, and function in addition to regulating lipid metabolism in the liver.	Carrots and green leafy vegetables (such as spinach) or retinyl esters from rich animal sources like eggs, fish, and liver
Choline **[31, 32]**	altering mitochondrial function, raising oxidative and endoplasmic reticulum (ER) stress, reducing the export of very-low-density lipoproteins (VLDL), and adversely influencing epigenetic processes such as DNA methylation	Animal meats and eggs
Polyphenols **[33,34]**	reduced de novo lipogenesis (via SREBP-1c), Regulate insulin sensitivity and adipokine modulation, and increased FA β-oxidation activity	In tea, coffee, red wine, beer, berries, nuts, whole grains, and fruits
Coffee **[35]**	antifibrotic, anti-inflammatory, and antioxidant qualities	two to three cups of filtered coffee without sugar

Iron **[36]**	encouraging oxidative stress by producing toxic free radicals through the Fenton reaction, which results in lipid peroxidation and cellular damage	Swiss chard, spinach, lentils, chickpeas
Copper **[37, 38]**	Excessive copper upregulating nuclear factor erythroid 2-related factor 2 (Nrf2) and activating and inhibiting the specific protein 1 (SP1)/Fyn pathway via metal-regulated transcription factor 1 (MTF-1), hepatic lipotoxicity is produced.	Meat, eggs, seafood, legumes
Zinc **[39]**	anti-oxidant, anti-inflammatory, and apoptotic effects	legumes (like chickpeas, lentils, and beans), nuts and seeds (such as pumpkin, hemp, and sunflower seeds), and certain vegetables (including spinach, mushrooms, and peas)

Conclusion

A key factor in the development and course of NAFLD is dietary makeup. Through increased de novo lipogenesis, insulin resistance, and oxidative stress, an excessive intake of macronutrients such trans fats, saturated fats, and refined carbs cause hepatic steatosis. On the other hand, balanced intake of lean protein sources, polyunsaturated fatty acids, and complex carbohydrates promotes metabolic equilibrium and lessens the buildup of liver fat. By regulating inflammation, oxidative stress, and insulin signalling, micronutrients such as vitamin E, vitamin D, zinc, and iron have protective effects; deficits in these components may worsen the severity of disease. Furthermore, while following nutrient-rich dietary models like the Mediterranean diet shows positive results, bad dietary habits including consuming large amounts of processed foods, sugar-sweetened beverages, and sedentary lifestyle patterns increase the risk. When combined, improving intake of macro and micronutrients and adopting healthy eating habits offer a sustainable and affordable approach to controlling and preventing NAFLD.

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5. Synthesis, characterization and applications of *Vinca rosea* derived nanoparticles as plant growth promoter and biocontrol of *Fusarium oxysporum ciceris*

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Abstract

Plants such as *Vinca rosea* are rich in bioactive compounds like alkaloids and flavonoids which can aid in nanoparticle synthesis while enhancing their bioactivity. These biologically synthesized nanoparticles can have promising antifungal, antimicrobial and plant growth- promoting properties, making them potential candidates for biocontrol and sustainable agriculture. Iron oxide (Fe_2O_3) nanoparticles were synthesised from *Vinca rosea* by co- precipitation method and had size of 5.10nm as confirmed by DLS with polydispersity index of 0.255. Further characterization was done by Fourier transformed infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM). Thermogravimetric analysis showed, weight loss of 36% at temperatures ranging between 25°C to 700°C. Antifungal activity was observed against *Fusarium oxysporum ciceris* (FOC), pathogen of chickpea plant which may cause losses upto 60-90%. Antifusaric activity of Fe_2O_3 nanoparticles was confirmed by spread plate technique and there was a decrease in fungal growth and biomass compared to control, confirmed by SEM and MDA assay. Nanoparticles lead to support seed germination and growth. Defense markers studyconfirmed it's potential in controlling the pathogen and promoting the growth.

Key words: Iron oxide nanoparticle, plant growth promoter, biocontrol, vinca rosea

1.Introduction

Nanotechnology, often described as the "technology at the nanoscale, involves the application of matter within the size range of 1–100 nm" (Ramsden, 2016). Unique chemical, physical and biological properties are contributed by nanoparticles because of its size (Thakker *et al*., 2012). In agriculture, nanotechnology is gaining increasing attention due to its potential to overcome the limitations of conventional practices, particularly the inefficient use of fertilizers and pesticides that often contaminate ecosystems (Pramanik *et al*., 2020, Baruah & Dutta, 2009). Nanoparticles, because of long shelf life, smaller size, enhanced efficiency and large surface area, offer opportunities for developing nanofertilizers and nanopesticides that can improve nutrient delivery and protect crops from diseases at lower dosages (Toksha *et al*., 2021, Babu *et al*., 2022).

Among the various nanomaterials, Fe_2O_3 NPs are of interest due to its higher availability in nature, structural diversity, and magnetic properties (Ogbezode *et al*., 2023, Machala *et al*., 2011). These nanoparticles can be synthesized using various approaches (Laurent *et al*., 2008, Álvarez-Chimal & Arenas-Alatorre, 2023). Physical methods, while advanced, often lacks control over particle size, whereas chemical methods allow precise regulation of particle dimensions and composition but may have and issue of toxic reagents (Cuenya, 2010). In contrast, green synthesis using plant based materials has emerged as a sustainable and cost-effective alternative. Plant biomolecules such as carbohydrates, flavonoids, and phenolics serve as stabilizing agents as well as reducing agents, leading to diverse morphologies under mild reaction conditions (Narayanan & Sakthivel, 2010). Dhandhukia *et al*. (2012) used *Fusarium oxysporum* f.sp. *cubense* to rapidly produce silver NPs, which were protein-capped (10–100 nm) and showed antimicrobial activity, indicating their potential antifungal application.

Chickpea (*Cicer arietinum*), serves as the "poor man's meat," is a vital pulse crop cultivated across Afro-Asian regions and serves as a major source of vitamins, minerals, plant based protein, and energy for millions of people (Muehlbauer *et al*., 2017, Yegrem, 2021). In India, chickpea contributes nearly 75% of total pulse production, highlighting its nutritional and economic importance (Joshi *et al*., 2001). However, chickpea productivity is severely constrained by Fusarium wilt, a disease caused by FOC. This soil-borne pathogen follows a triphasic life cycle and colonizes the xylem, ultimately leading to wilt conditions and untimely senescence of the plants (Bhar *et al.*, 2021) followed by death. Annual crop losses due to such diseases are substantial, ranging between 10–40% (Patel *et al.*, 2024). Hence, there is a critical need for sustainable strategies to protect chickpea crops from fungal pathogens.

Vinca rosea, a medicinal plant widely recognized in both traditional and modern medicine, presents a promising candidate for ecofriendly synthesis of nanoparticles. V. rosea produces around 130 alkaloids, including vinblastine and vincristine, which are well-known anticancer agents, along with several other bioactive compounds with antimicrobial, antioxidant, and antimutagenic properties (Al-Azzawi *et al.*, 2023, Rajashekara, S., & Baro, 2022). These phytochemicals not only contribute to therapeutic applications but also play a vital role in nanoparticle synthesis by reducing and stabilizing metal ions.

In this context, the present study focuses on the green synthesis of Fe_2O_3 NPs using *Vinca rosea* extract and their evaluation against FOC.

2. Materials and Methods

2.1 Extract preparation

Leaves of *Vinca rosea* were collected from the CHARUSAT campus (Geolocation: 22.6020241, 72.8177446). The leaves were dried overnight at 45°C and grinded using mixture. 5 grams of the powder was mixed with 200 ml of deionized glass distilled water and boiled at 80°C for 1 hour using a hot plate with a magnetic stirrer. The resulting extract was first filtered through standard filter paper, followed by filtration with filter paper no.1 (Whatmann). The synthesized *V. rosea* extract was stored at 4°C for use in nanoparticle synthesis (Bibi *et al.*, 2019).

2.2 Fe_2O_3 NPs synthesis and quantification

Fe_2O_3 NPs were synthesized using ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) at a 0.2 M concentration and ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) at 0.1 M, maintaining a 2:1 molar ratio. Salt solution and plant extract in ratio of 1:1 was stirred at room temperature for 30 minutes using a magnetic stirrer. Dark black precipitates developed when pH was raised using 0.1 M NaOH indicating NPs formation. The precipitate was collected by centrifugation at 6000 rpm for 15 minutes, followed by rinsing with deionized distilled water and twice with ethanol and drying (Bibi *et al*., 2019). Precipitates obtained were quantified using gravimetric analysis

2.3 Characterization of synthesized Iron Oxide Nanoparticles

2.3.1 UV visible Spectrophotometric analysis

For UV visible spectrophotometric analysis, the sample was prepared by dissolving 10mg of synthesized nanoparticles in distilled water and exposed to probe sonication for 25- 30 min followed by scanning in the range of 200nm to 800 nm.

2.3.2 Dynamic light scattering (DLS)

For DLS, the synthesized NPs (stock solution 10mg/ml) was suspended in distilled water and then exposed to probe sonication for 5-10 minutes for dissolution of NPs. DLS gave information about the dispersity index and z-average size of NPs (Yang *et al*., 2014).

2.3.3 Scanning electron microscopy (SEM)

For SEM analysis, nanoparticles were allowed to remove moisture in a hot air oven overnight at 50°C. The morphology of nanoparticles was observed by SEM images at 700X and 2000X (Abomughaid *et al*., 2023).

2.3.4 Fourier-transform infrared spectroscopy (FTIR)

Samples were ground with KBr to create a fine powder, which was then pressed into a thin pellet. This pellet was analyzed using an FTIR (Thermo Scientific NICOLET FTIR 6700). Spectra were obtained as % transmission against wavenumber (Abomughaid *et al*., 2023).

2.3.5 Thermogravimetric analysis (TGA)

The stability of iron oxide nanoparticles at different temperatures was investigated using thermogravimetric analysis (TGA). For this, the dried nanoparticles were placed into the instrument, with the temperature range set between 25°C to 900°C (Sinhamahapatra *et al*., 2012)

2.4 Applications

2.4.1 Antifusaric activity

2.4.2 Well diffusion assay

Fungal (FOC) spore suspension 10^6 spores/ml (100 µl) was spread on a PDA plate. The 5mm well was punched and in that 100µl NPs (5ppm, 10ppm, 50ppm, 100ppm) solution was added. The control well had 100µl of distilled water. Then the plates were incubated for 29-30°C (Rathod *et al*., 2023).

2.4.3 Spread plate technique

Fungal suspension which contained 10^6 spores /ml from which 100 µl of the solution was taken and 100 µl nanoparticle solution was taken of different concentration (5ppm,10ppm,50ppm,100ppm) and each mixture was incubated for 1 hour and was spread on Potato Dextrose Agar (PDA) plate (Patel *et al*., 2024).

2.4.4 Broth assay

Suspension of *Fusarium oxysporum ciceris* 100 µL spores (10^6 spores/mL) and 100 µL of each of the four nanoparticle dilutions (5 ppm, 10 ppm, 50 ppm, and 100 ppm) were added to potato dextrose broth. The mixture was then incubated at 28°C for 7 days. After growth, the broth was filtered through Whatman filter paper No. 1, and the fungal mat was analysed using gravimetric method (Thakker *et al*., 2023).

SEM Analysis

Into a 100 ml PDB having100µl of fungal spores (10^6 spores/mL) and 100µl of different concentrations of NPs (5ppm, 10ppm, 50ppm, 100ppm) were added, followed by a 9-day incubation period at 30°C. SEM (JEOL JSM- 6010LA) with a 10 kV voltage was used to analyze morphologically, reduction of FOC growth in the presence of the NPs (Rathod *et al*., 2023).

Malondialdehyde (MDA) content

Fungal mat was centrifuged after crushing it using Three milliliters of 0.1 M sodium phosphate buffer (pH 7). 500 µl of 10% trichloroacetic acid (TCA) and 500 µl of 0.335% (w/v) thiobarbituric acid (0.335 g in 100 ml of glacial acetic acid) were added to 500 µl of the mycelial homogenate. This mixture was incubated in a hot water bath for one hour (Chouhan *et al*., 2023). Absorbance was measured at 532, 450, and 600 nm. MDA was calculated using the following formula (Eq1) (Zahedi *et al*., 2023).

MDA (µM/g)= $6.45(OD_{532}-OD_{600})-0.56\ OD_{450}$-- Eq (1)

2.5 Effect on seed germination and vegetative parameters

2.5.1 Water Agar test

Sterilized seeds were treated with different concentration (5ppm, 10ppm, 50ppm, 100ppm) of nanoparticles and different concentration (5ppm, 10ppm, 50ppm, 100ppm) of nanoparticles mixed with FOC suspension for 20h. Seeds were transferred on 1.5% agar plate and incubated at 30°C for 4-5 days. Seed germination rate and the radical length of germinated seeds were also measured.

Germination% = Number of seeds germinated *100/Total numbers of seeds-------- Eq (2)

Vigor index = Germination% * average length of seedlings ---------------------------Eq (3)

2.5.2 Pot studies

For assessing PGP activity, sterilized seeds were soaked in distilled water (control) or in different concentrations of NPs (5 ppm, 10 ppm, 50 ppm, and 100 ppm) for 20 hours before sowing in autoclaved soil-filled plastic pots. For biocontrol activity, sterilized seeds were soaked in FOC suspension (control) or in FOC combined with varying concentrations of nanoparticles (5 ppm, 10 ppm, 50 ppm, and 100 ppm) for 20 hours and then sown in plastic pots containing autoclaved soil (Rathod *et al*., 2023)

2.5.3 Physiological stress marker assays

2.5.4 Estimation of Superoxide dismutase (SOD) from plants

Leaf tissue (0.5 g) was crushed in phosphate buffer (0.1 M, pH 6.0), centrifuged, and the supernatant was used as the source of enzyme Beauchamp and Fridovich (1971). The reaction mixture contained phosphate buffer, EDTA, methionine, NBT, riboflavin, and enzyme extract, with respective controls and blanks. After exposing the tubes to light for 20 minutes, absorbance was measured at 560 nm, and SOD activity was calculated as the percentage inhibition of NBT reduction.

2.5.4.2 Estimation of total phenolic compounds

For the assay, 1 mL of methanol-homogenized plant extract was taken in a test tube and mixed with 5 mL distilled water and 0.5 mL Folin–Ciocalteu's reagent, followed by thorough shaking. After 5 minutes, 1.5 mL of 20% sodium carbonate solution was added, and the volume was made up to 10 mL with distilled water. The reaction mixture was incubated at room temperature until a deep blue coloration developed, and absorbance was measured at 750 nm using a UV–Vis spectrophotometer. A standard curve was prepared with gallic acid, and the phenolic content was expressed as gallic acid equivalents (GAE). All analyses were performed in triplicate, with reagent blanks serving as controls

2.5.4.3 Estimation of Chlorophyll and Carotenoids from plant

Carotenoid and chlorophyll were estimated following Sumanta *et al*., (2014). Leaf tissue (0.5 g) was homogenized in 10 mL acetone, and the extract was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant (0.5 mL) was diluted with 4.5 mL solvent and absorbance was measured at 661.6, 644.8, and 470 nm using a spectrophotometer. Chlorophyll a, chlorophyll b, and carotenoids were calculated using the equations:

Chla = 11.24 A661.6 – 2.04 A644.8 ---------Eq. 4

Chlb = 20.13 A644.8 – 4.19 A661.6---------- Eq. 5

Carotenoids = (1000 A470 – 1.90 Ca – 63.14 Cb)/214 --------Eq.6

2.5.4.4 Estimation of L- proline content from plant

Fresh tissue (0.5 g) was homogenized in 3% sulfosalicylic acid, centrifuged, and the supernatant was used for analysis. The assay mixture contained equal volumes (1 ml) of extract, glacial acetic acid, and acid ninhydrin reagent, followed by incubation in a boiling water bath (100°C) for 1 h to develop a pink-red chromophore. Toluene (2 ml) was added and the organic phase was separated for absorbance measurement at 520 nm against toluene blank. Free proline content was quantified using a standard curve prepared with L-proline (5–100 μg/mL) and expressed as μg/g fresh weight Bates *et al*. (1973).

2.6 Statistical Analysis

All the experiments were in triplicates. For seed germination, pot trials also three replicates were kept and average of seeds in each plate and plants were taken. Further statistical significance was calculated using ANOVA

3. Results

Fig. 1: *Vinca rosea* derived Fe_2O_3 Nps

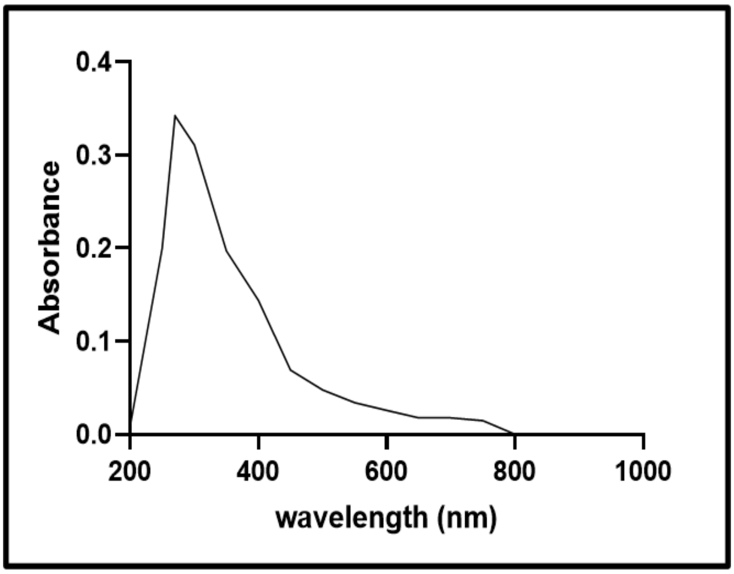


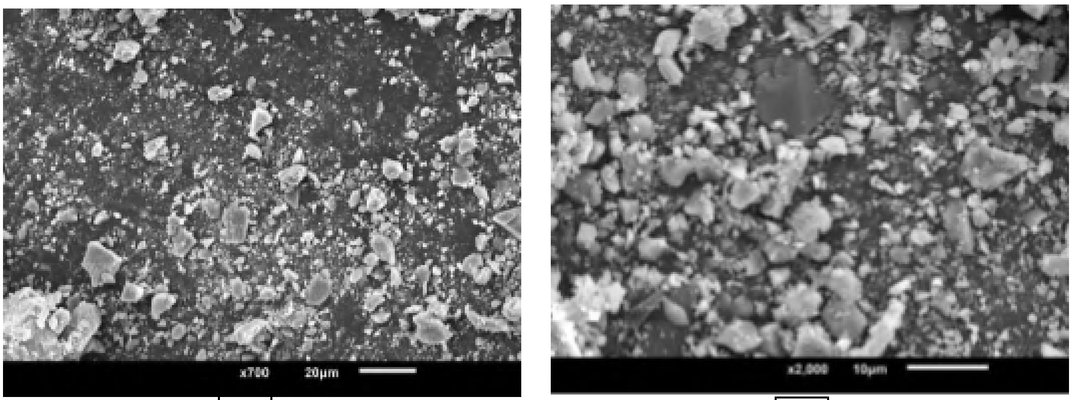
Fig. 2: UV visible spectra of Fe_2O_3 Nps

The UV–visible spectra analysis of Fe_2O_3 Nps (Figure 2) revealed an absorption peak at 250 nm.

Table 1: DLS of Fe_2O_3 Nps

Peak	Size (d.nm)	% Intensity	St Dev
Peak 1	5.783	72.8	1.03
Peak 2	2244	27.2	820.2

The average particle size of the Fe_2O_3 Nps was 5.103 nm having a polydispersity index (PDI) of 0.255 as shown in Table 1. The DLS profile exhibited two peaks, where Peak 1 represented well-dispersed nanoparticles, while Peak 2 indicated the presence of aggregates.



a b

Fig 3: SEM images of synthesized Iron oxide nanoparticles. 3a: showing irregular shape of Fe_2O_3 Nps, 3b: clusters of Fe_2O_3 Nps

SEM image at 700× magnification is shown in Figure 3a, while Figure 3b corresponds to 2000× magnification. The analysis revealed irregularly shaped nanoparticles forming clusters with rough surface morphology. The cluster sizes of the Fe_2O_3 Nps were observed to range between 100 and 200 nm.

Table 2: FTIR peaks and functional groups of iron oxide nanoparticles

Wavenumber (cm^{-1})	**Functional Group**	**Interpretation**
3551, 3414, 3141	O–H stretching (broad)	Indicative of hydroxyl groups (–OH) typically found in alcohols or phenols, possibly hydrogen-bonded.
2922, 2853	C–H stretching	Alkane C–H bonds, common in $-CH_2-$ and $-CH_3$ groups (symmetric and asymmetric stretching).
2031	C≡C or C≡N stretching	Possibly alkyne (–C≡C–) or nitrile (–C≡N) groups; however, this region can also show overtone/ combination bands.

1616	C=O stretching/C=C Stretching	Can correspond to carbonyl groups (C=O) in ketones, aldehydes, or conjugated C=C in aromatics.
1512	Aromatic C=C stretching	aromatic ring structures (benzene derivatives).
1384	C–H bending (methyl groups)	Symmetric bending in $–CH_3$ groups.
1033	C–O stretching	Typically seen in alcohols, ethers, esters, and carboxylic acids.
622, 479	Metal–O bond vibrations	Likely corresponds to Fe–O stretching, confirming presence of iron oxide nanoparticles.

In Figure 4, TGA was employed to evaluate the thermal stability of the biosynthesized iron oxide nanoparticles. The analysis revealed a gradual decrease in weight from 8.3 mg to 5.26 mg as the temperature increased from 25 °C to 700 °C. This corresponds to an overall weight loss of approximately 36%, which can be attributed to the thermal decomposition of biomolecules present on the nanoparticle surface. The initial weight reduction up to ~150 °C is likely due to the evaporation of adsorbed water molecules, followed by further weight loss between 150 °C and 400 °C, which may be associated with the degradation of organic residues or phytochemicals involved in nanoparticle synthesis. Beyond 400 °C, only a slight weight change was observed, indicating the formation of thermally stable crystalline iron oxide nanoparticles.

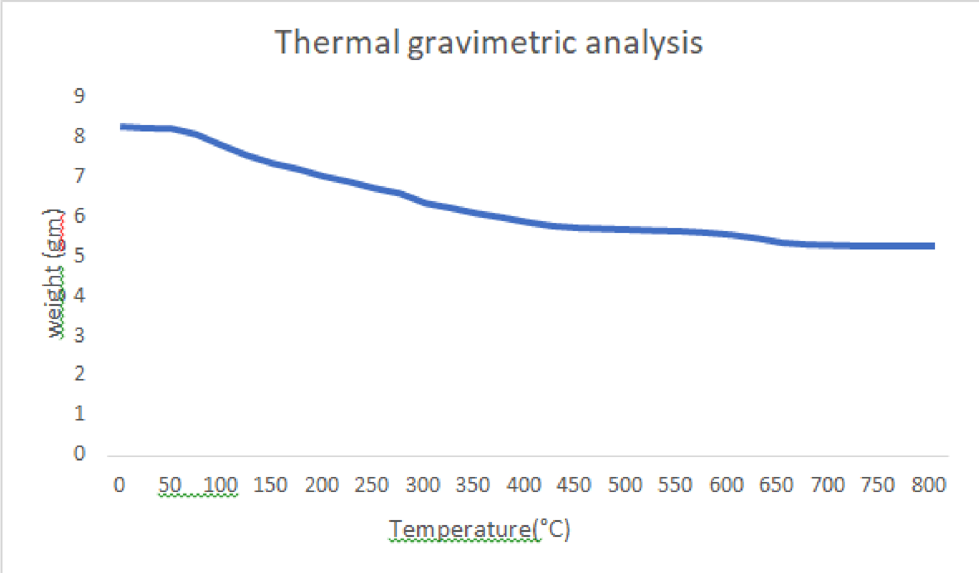


Figure 4: TGA of Fe_2O_3 Nps

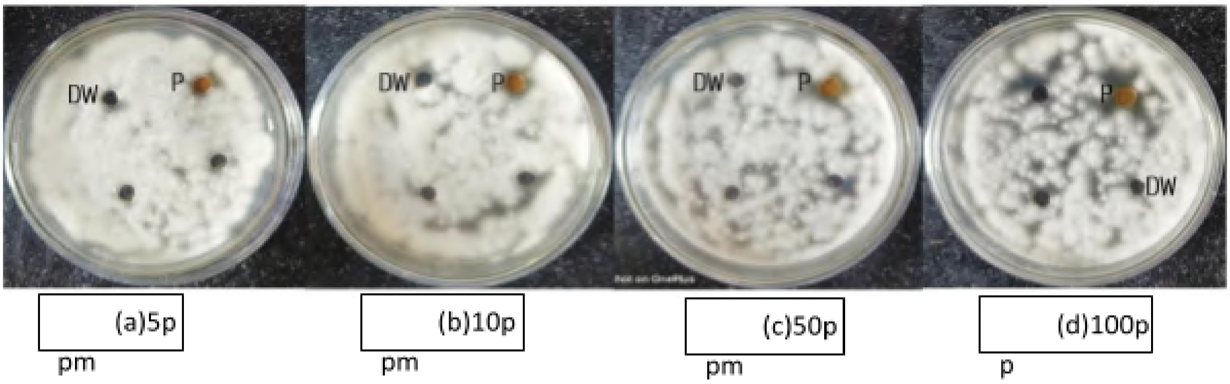


Figure 5: Well diffusion assay showing the antifungal activity of biosynthesized iron oxide nanoparticles at different concentrations: (a) 5 ppm, (b) 10 ppm, (c) 50 ppm, and (d) 100 ppm. 'DW' represents the distilled water control, while 'P' represents the positive control.

In above Figure 5, In Antifungal activity there was no proper zone of inhibition was observed against FOC, but with increasing concentration of NPs there is decrease in fungal growth.

Treatment	Mat weight (gm)
Control	2.64± 0.10
5ppm NPs	2.33±0.04^{ns}
10ppm NPs	2.18±0.6^{ns}
50ppm NPs	1.49±0.16**
100ppm NPs	1.12±0.10***

Table 3: Quantitative analysis of fungal mat using broth assay

(a)FOC	(b)5ppm +FOC	(c)10ppm +FOC	(d)50ppm +FOC	(e)100ppm +FOC

Figure 6: Antifungal activity of biosynthesized iron oxide nanoparticles against *Fusarium oxysporum* f. sp. *cubense* (FOC) using the co-culture technique. Treatments included: (a) FOC control, (b) 5 ppm + FOC, (c) 10 ppm + FOC, (d) 50 ppm + FOC, and (e) 100 ppm + FOC.

From the results of spread plate technique obtained, it was noted that with increasing concentration of nanoparticles, there is a decrease in fungal growth, and NPs give antifungal activity against FOC. ns (p Value greater than 0.05) nonsignificant as compared to control. * (p Value between 0.05 and 0.01) significant at 5 % as compared to control. ** (p Value between 0.01 and 0.001) significant at 1 % as compared to control. *** (p Value less than 0.001) significant at 0.1 % as compared to control

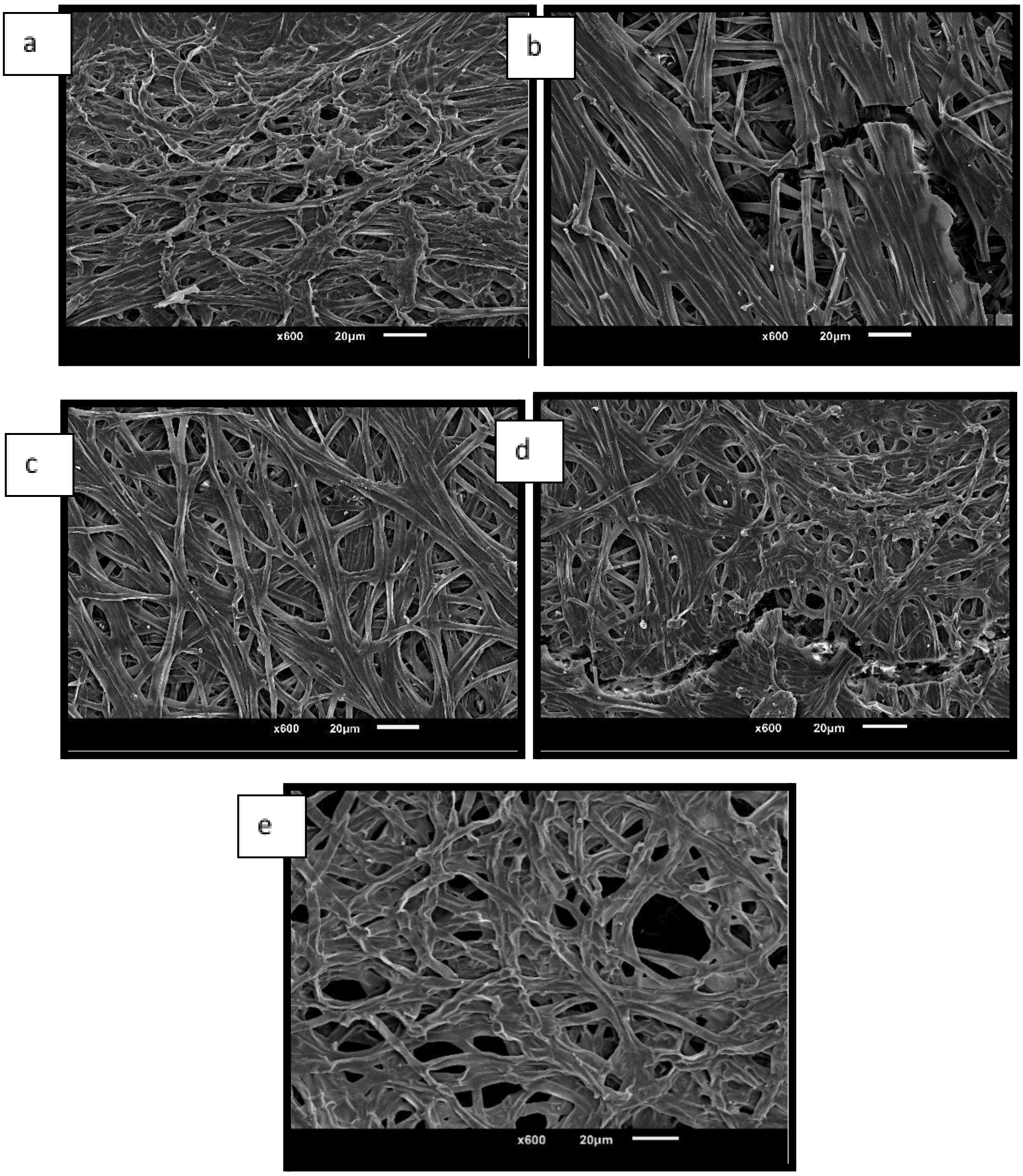


Figure 7: SEM image of FOC mat after 10 days (a)FOC (b)5ppm+FOC (c)10ppm+FOC (d)50ppm+FOC (e)100ppm+FOC

A reduction in fungal mat formation was observed with increasing concentrations of the nanoparticle (NP) solution. In comparison to the control (Figure 7a), a slight disruption of the fungal structure is evident at 5 ppm (Figure 7 b) and 10 ppm (figure 7 c). As the NP concentration increases, the extent of fungal degradation becomes more pronounced. Notably, in Figures (7 d) and (7 e), a substantial breakdown of the fungal mat is observed, along with a visible increase in the spacing between the fungal structures.

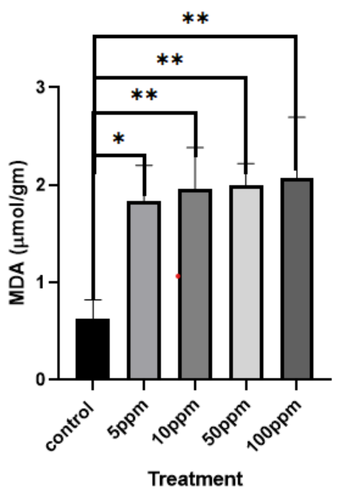


Figure 8: Malondialdehyde analysis of FOC at different concentration (5ppm, 10ppm, 50ppm, 100ppm) of NPs. * (p Value between 0.05 and 0.01) significant at 5 % as compared to control. ** (p Value between 0.01 and 0.001) significant at 1 % as compared to control.

Based on the figure 8, the sample exhibits lower malondialdehyde (MDA) content compared to the others, indicating minimal fungal-induced oxidative damage. However, as the concentration of nanoparticles (NPs) increases, a corresponding rise in MDA levels is observed, suggesting enhanced lipid peroxidation and greater fungal damage with higher NP concentrations

In the biocontrol treatment (Figure 9a, Table 3), seeds challenged with *Fusarium oxysporum* f. sp. *cubense* (FOC) exhibited reduced growth, whereas nanoparticle-treated seeds showed a marked increase in germination and radical elongation. At higher concentrations (50 ppm and 100 ppm), a 100% germination rate was recorded, along with a noticeable increase in radical length and a slight improvement in plumule length at 100 ppm. Similarly, in the PGP treatment (Figure 9b, Table 3), nanoparticle application alone resulted in improved germination and seedling growth compared to the control, with the highest vigor index and 100% germination observed at 100 ppm. Overall, the assay demonstrated that iron oxide nanoparticles enhance seed germination and seedling vigor both by suppressing the effects of FOC (biocontrol activity) and by stimulating growth under normal conditions (PGP activity).

As shown in Table 3, the biosynthesized iron oxide nanoparticles enhanced seed germination, radical length, and vigor index under both biocontrol (FOC-challenged seeds) and plant growth-promoting (PGP) conditions.

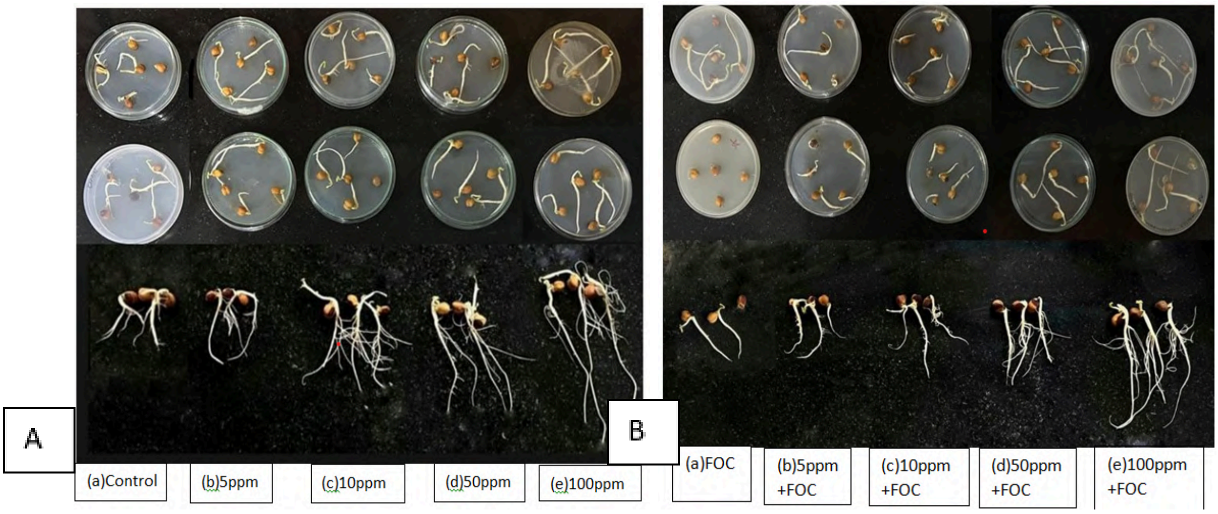


Figure 9: Water agar test for seed germination showing the effect of biosynthesized iron oxide nanoparticles. (A) Biocontrol treatment: seeds inoculated with *Fusarium oxysporum* f. sp. *cubense* (FOC) and treated with different concentrations of nanoparticles (5, 10, 50, and 100 ppm). (B) Plant growth-promoting (PGP) treatment: seeds treated with nanoparticles at concentrations of 5, 10, 50, and 100 ppm without pathogen challenge.

Table 3: The water agar test was conducted to evaluate seed germination under the combined effect of biocontrol and PGP treatments

Experiment	Seed treated	% Germination	Radical length (cm)	Vigor index
Biocontrol	FOC	40%	3.45±3.45	138.0
Biocontrol	5 ppm + FOC	80%	7.12±0.32ns	569.6
Biocontrol	10 ppm + FOC	90%	7.95±1.45ns	555.5
Biocontrol	50 ppm + FOC	100%	11.98±2.68**	1198.0
Biocontrol	100 ppm + FOC	100%	12.19±0.95**	1219.0
PGP	Control	100%	4.77±0.57	477.0

PGP	5 ppm	90%	9.22±1.22ns	829.8
PGP	10 ppm	90%	9.54±4.1ns	858.6
PGP	50 ppm	80%	11.1±0.9*	888.0
PGP	100 ppm	100%	12.35±1.55**	1235.0

Data are expressed as mean ± standard deviation; ns = non-significant, * = significant, ** = highly significant compared to control.

Table 4: Comparative effect of iron oxide nanoparticles on vegetative growth parameters of chickpea under biocontrol (with FOC) and PGP treatments

Parameters	**Biocontrol (FOC)**	**PGP Treatment**
No. of branches	Control: 7.90 ± 0.54 5 ppm: 7.21 ± 4.50 ns 10 ppm: 8.87 ± 1.85 ns 50 ppm: 8.93 ± 0.96 ns 100 ppm: 9.33 ± 1.05 ns	Control: 9.83 ± 0.61 5 ppm: 9.80 ± 0.86 ns 10 ppm: 8.97 ± 1.32 ns 50 ppm: 10.07 ± 1.33 ns 100 ppm: 9.00 ± 1.77 ns
No. of leaves	Control: 106.85 ± 5.97 5 ppm: 95.28 ± 63.69 ns 10 ppm: 113.33 ± 26.80 ns 50 ppm: 118.67 ± 19.80 ns 100 ppm: 126.60 ± 11.43 **	Control: 118.10 ± 19.85 5 ppm: 122.20 ± 23.60 ns; 10 ppm: 132.08 ± 4.50 *** 50 ppm: 134.30 ± 15.02 *** 100 ppm: 136.60 ± 14.60 ***
No. of root hairs	Control: 8.71 ± 0.50 5 ppm: 9.89 ± 5.36 ns 10 ppm: 11.93 ± 1.94 ns 50 ppm: 32.67 ± 3.74 ** 100 ppm: 34.33 ± 3.54 ***	Control: 25.23 ± 4.34 5 ppm: 28.72 ± 3.26 ns; 10 ppm: 28.27 ± 4.83 ns 50 ppm: 33.53 ± 3.83 ns 100 ppm: 34.27 ± 4.33 *
Total length (cm)	Control: 6.69 ± 2.37 5 ppm: 24.37 ± 13.86 * 10 ppm: 30.84 ± 2.53 ** 50 ppm: 32.97 ± 2.39 *** 100 ppm: 35.91 ± 3.60 ***	Control: 27.20 ± 3.29 5 ppm: 29.13 ± 2.05 ns 10 ppm: 33.69 ± 3.56 ns 50 ppm: 36.13 ± 4.34 * 100 ppm: 38.42 ± 4.38 **

Shoot length (cm)	Control: 15.53 ± 2.61 5 ppm: 17.30 ± 9.17 ns 10 ppm: 21.79 ± 2.26 ns 50 ppm: 22.91 ± 1.97 ns 100 ppm: 25.39 ± 2.83 ns	Control: 18.60 ± 2.96 5 ppm: 19.88 ± 0.54 ns 10 ppm: 23.08 ± 2.68 ns 50 ppm: 24.39 ± 3.62 ns 100 ppm: 24.83 ± 2.51 ns
Root length (cm)	Control: 22.22 ± 0.46 5 ppm: 7.25 ± 4.18 ns 10 ppm: 9.05 ± 0.46 ns 50 ppm: 10.06 ± 0.64 ns 100 ppm: 10.52 ± 0.99 ns	Control: 8.63 ± 0.57 5 ppm: 9.25 ± 0.56 ns 10 ppm: 10.61 ± 0.99 ns 50 ppm: 11.74 ± 1.07 ns 100 ppm: 13.59 ± 2.17 ns
Total weight (g)	Control: 0.64 ± 0.20 5 ppm: 0.70 ± 0.39 ns 10 ppm: 0.84 ± 0.13 ns 50 ppm: 0.90 ± 0.18 ns 100 ppm: 1.01 ± 0.19 ns	Control: 0.80 ± 0.18 5 ppm: 0.85 ± 0.13 ns 10 ppm: 1.10 ± 0.22 ns 50 ppm: 1.12 ± 0.24 ns 100 ppm: 1.06 ± 0.19 ns
Shoot weight (g)	Control: 0.58 ± 0.18 5 ppm: 0.61 ± 0.34 ns 10 ppm: 0.76 ± 0.11 ns 50 ppm: 0.78 ± 0.17 ns 100 ppm: 0.87 ± 0.17 ns	Control: 0.72 ± 0.17 5 ppm: 0.72 ± 0.14 ns 10 ppm: 0.90 ± 0.18 ns 50 ppm: 0.91 ± 0.18 ns 100 ppm: 0.91 ± 0.16 ns
Root weight (g)	Control: 0.05 ± 0.01 5 ppm: 0.09 ± 0.04 ns 10 ppm: 0.08 ± 0.03 ns 50 ppm: 0.12 ± 0.04 ns 100 ppm: 0.14 ± 0.06 ns	Control: 0.06 ± 0.02 5 ppm: 0.13 ± 0.04 ns 10 ppm: 0.20 ± 0.11 ns 50 ppm: 0.21 ± 0.09 ns 100 ppm: 0.15 ± 0.06 ns

p-value has been calculated using two-way ANOVA and its interpretation as follow: ns (p Value greater than 0.05) nonsignificant as compared to control. * (p Value between 0.05 and 0.01) significant at 5 % as compared to control. ** (p Value between 0.01 and 0.001) significant at 1 % as compared to control. *** (p Value less than 0.001) significant at 0.1 % as compared to control. All the samples were taken in triplicates

Vegetative parameters of chickpea plants were assessed after 28 days of pot trials to evaluate the efficiency of iron oxide nanoparticles under in vivo conditions. Seeds were pre-soaked in nanoparticle and fungal suspensions, and the subsequent growth was compared with respective controls. In the biocontrol assay, plants exposed to different concentrations of nanoparticles along with *Fusarium oxysporum* f. sp. *ciceri* (FOC) showed improved vegetative growth compared to those treated with FOC alone, confirming the nanoparticles' biocontrol activity. Enhanced root length was a prominent observation, indicating better mineral and nutrient absorption from the soil, which directly contributed to higher plant vigor. In the pot trials assay, nanoparticle-treated chickpea plants also exhibited superior growth compared to the distilled water control. The presence of nanoparticles notably promoted root development, facilitating nutrient uptake and thereby improving overall plant performance. These findings demonstrate that iron oxide nanoparticles function dually as biocontrol agents against fungal pathogens and as plant growth-promoting (PGP) agents under natural conditions.

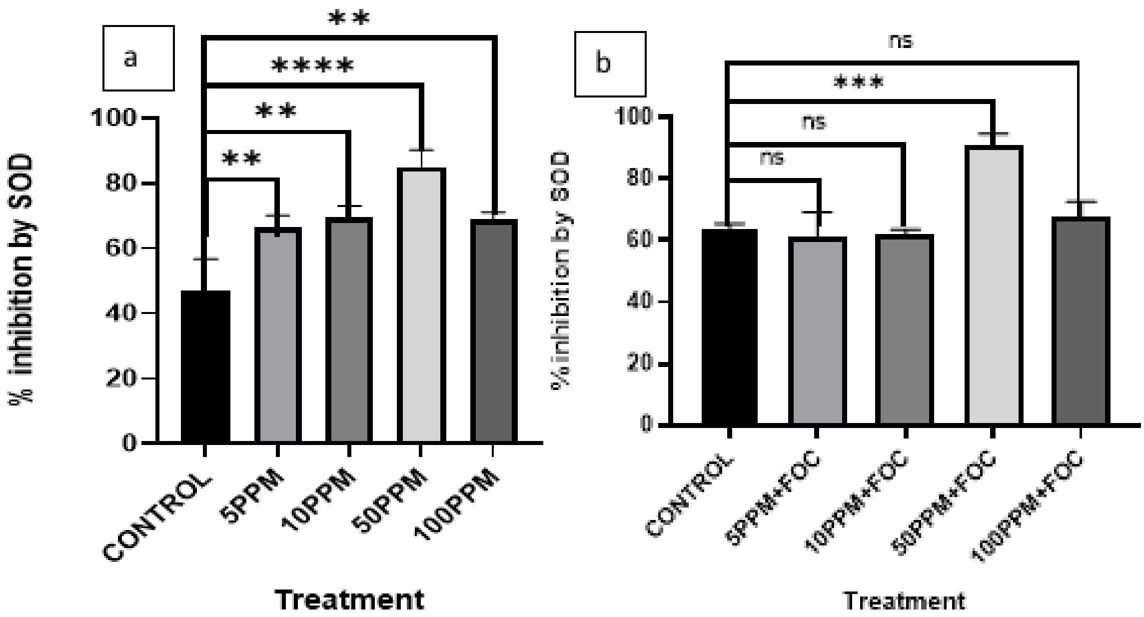


Figure 10: Inhibition of SOD activity in presence of nanoparticle concentrations a) Nanoparticles with different concentration b) nanoparticle with different concentrations with FOC

In the biocontrol assay (Figure 8a), where the control was FOC and treatments included varying concentrations of nanoparticles combined with the fungus, the highest SOD activity was observed at 50 ppm + FOC. This concentration showed a significant increase compared to the control and other treatments, highlighting the enhanced efficiency of nanoparticles in stimulating SOD production. Interestingly, at 100 ppm, the activity did not continue to rise and instead exhibited a slight decline. Similarly, (Figure 8 b), where untreated plants served as the control, SOD activity displayed a dose-dependent increase with nanoparticle treatment, peaking at 50 ppm. However, at the highest concentration (100 ppm), a decline in activity was again noted. These findings suggest that iron oxide nanoparticles effectively enhance SOD activity up to an optimal concentration (50 ppm), beyond which the effect diminishes.

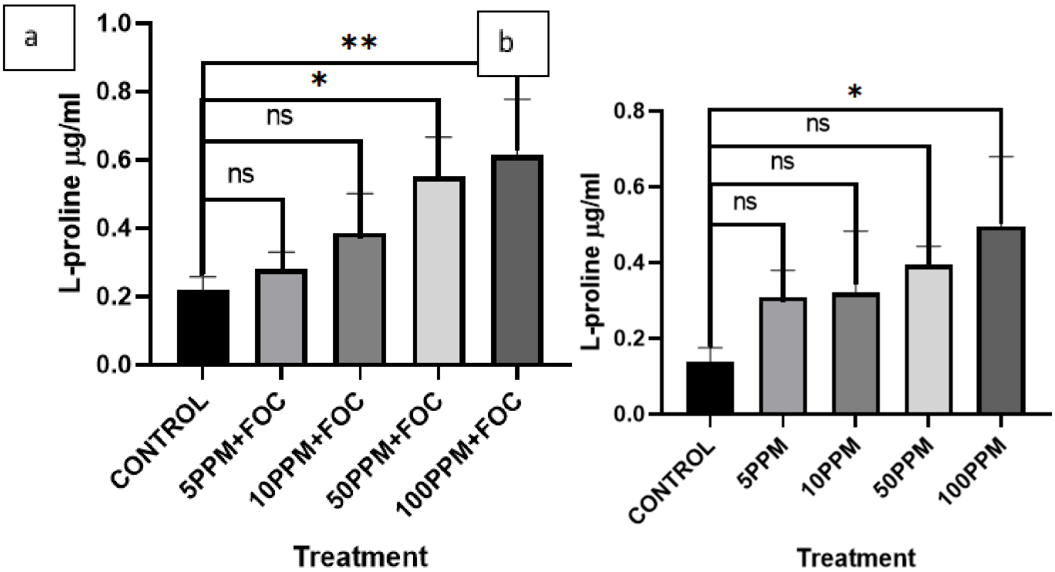


Figure 11 Changes in proline content a) treatment with nanoparticles in presence of FOC b) comparison of control and nanoparticle treatment on proline content

L-proline accumulation increased significantly with rising nanoparticle concentrations. SOD is a metalloenzyme that plays a crucial role in cellular defense by catalyzing the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen, thereby protecting cells from oxidative stress during biotic and abiotic challenges In Figure 11(a), the combination of FOC and nanoparticles enhanced proline levels, Similarly, Figure 11 (b) shows that even without FOC, higher nanoparticle concentrations induced proline accumulation, highlighting their independent role in stress adaptation.

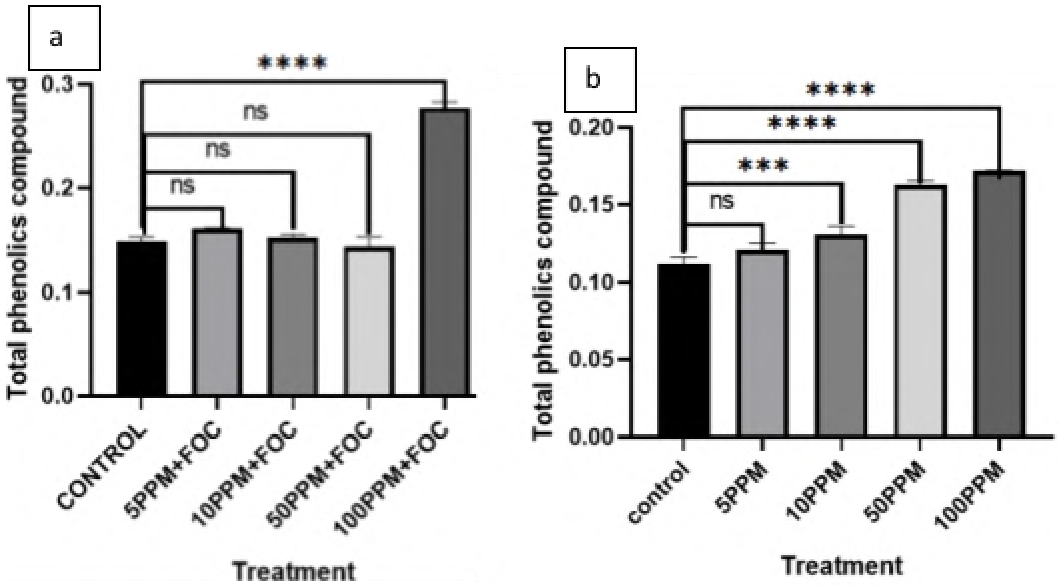


Figure 12 Changes in total phenolics content a) treated with different concentration of nanoparticles with FOC b) with different concentration of nanoparticles

Phenol accumulation increased significantly with nanoparticle treatment. In Figure 12 (a), seeds treated with FOC + nanoparticles showed higher phenolic and lignin levels than FOC alone, with the maximum accumulation at 100 ppm, indicating enhanced lignin synthesis and improved plant defense against infection. Similarly, in Figure 12 (b), nanoparticle-treated plants displayed a dose-dependent rise in phenolics compared to untreated controls, with the highest accumulation at 100 ppm, suggesting stronger protection against pathogens.

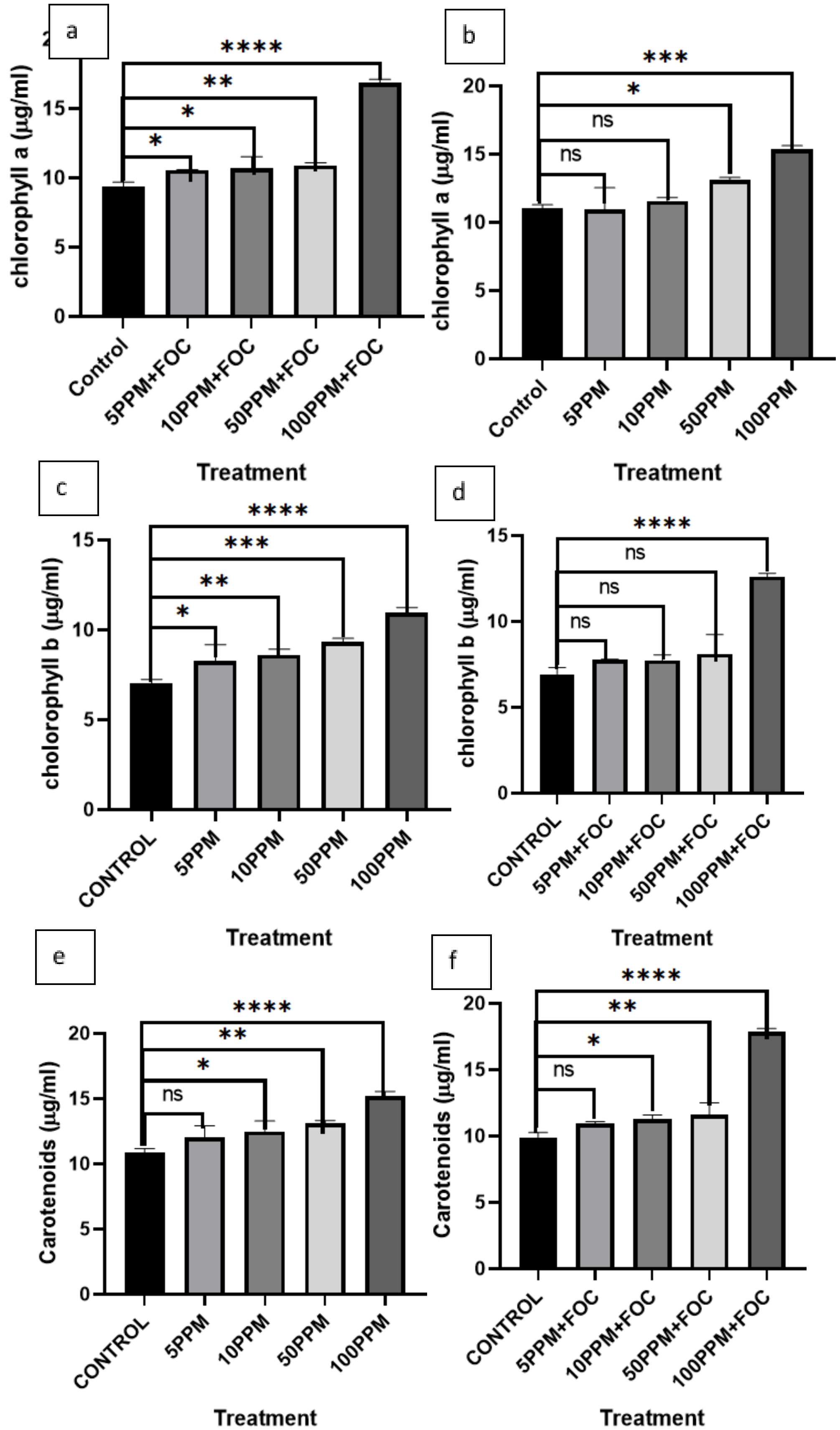


Figure 13. Changes in Chlorophyll a concentration a) Nanoparticles of different concentration + FOC and b) nanoparticles of different concentrations Chlorophyll b concentration c) nanoparticles with different concentration d) nanoparticles of

different concentration +FOC Carotenoid concentration e) nanoparticles with different concentration f) nanoparticles with different concentration + FOC

In Figure 13 (a) and (b), chlorophyll a content was found to be higher in 100 ppm and 100 ppm + FOC treatments compared to the control, with maximum accumulation observed at these concentrations, which may help in preventing chlorosis in plants. A similar trend was observed for chlorophyll b in Figure 13 (c) and (d), where both 100 ppm and 100 ppm + FOC treatments exhibited higher accumulation than the control, again showing maximum levels at the same concentrations. Carotenoids, as shown in Figure 13 (e) and (f), also increased progressively with rising nanoparticle concentrations, with the highest accumulation recorded at 100 ppm and 100 ppm + FOC, indicating that nanoparticles enhance pigment content in plants.

4. Discussion

Fe_2O_3 NPs were synthesized via a green route using *Vinca rosea* leaf extracts at room temperature (Fig. 1). The UV–visible absorption spectra of the synthesized NPs (Fig. 2) exhibited a peak at 250 nm, whereas Eldeeb *et al*. (2023) reported a characteristic absorption at 259 nm. This slight variation in peak position can be ascribed to differences in the phytochemical constituents of the respective plant extracts. The morphology of the Fe_2O_3 NPs, examined through SEM analysis, revealed particles ranging in size from 100 to 200 nm, with irregular shapes and a tendency to form agglomerates (Fig. 3a, 3b). Similar observations were made by Lohrasbi *et al*. (2019), who reported agglomeration of Fe_2O_3 nanoparticles synthesized using *Plantago major* leaf extract, attributing it to the sticky nature of iron during green synthesis. Due to H-bonding present in bioactive molecules, the particles appeared to be in the form of aggregates (Bibi *et al*., 2017a, Bibi *et al*., 2017b, Nazar *et al*., 2017). It is noteworthy that aggregates of smaller nanoparticles are often detected as single larger particles in DLS measurements. Consistent with previous studies, Fe_2O_3 NPs also demonstrated a tendency to form larger clusters under aqueous conditions (Yang *et al*., 2014). The characteristic bands at 622 and 479 cm^{-1} confirmed Fe–O vibrations and successful nanoparticle formation. These findings are in agreement with Bouafia and Laouini (2020), who reported similar functional groups during the green synthesis of Fe_2O_3 nanoparticles using *Mentha pulegium* extract, with minor variations attributed to differences in phytochemical composition of the plant extract. The TGA analysis of biosynthesized Fe_2O_3 nanoparticles (Fig. 4) showed a weight reduction from 8.3 mg to 5.26 mg as the temperature reaches to 700°C, corresponding to ~36% weight loss. The initial loss was due to the evaporation of bound water molecules, while the subsequent loss resulted from the decomposition of biomolecules acting as stabilizers or capping agents. Similar two-step weight losses have been reported by Sinhamahapatra *et al*. (2012), and the overall 10–26% loss observed indicates good thermal stability of the nanoparticles.

In the spread plate technique, a nanoparticle concentration dependent inhibition of fungal growth was observed, where higher NP concentrations significantly reduced colony formation compared to the control (fig 6), consistent with earlier findings that plant-mediated Fe_2O_3 NPs exhibit strong antifungal effects against *Fusarium* species (Parseghian *et al*., 2024, Elsherbiny & Omar, 2023). SEM analysis of fungal mats further confirmed structural disruption, showing slight hyphal disintegration at 5–10 ppm and severe breakdown at 50–100 ppm (fig 7 a-e). MDA content increased progressively with NP concentration (fig 8), indicating elevated lipid peroxidation and oxidative stress, which aligns with the mechanism of reactive oxygen species (ROS) generation by Fe_2O_3 NPs leading to cell membrane damage (Benamara *et al*., 2025). Similarly, fungal mat weight decreased significantly at higher NP levels, demonstrating suppressed biomass accumulation, as also reported with ZnO NPs against *Fusarium* (Gonzalez-Merino etal., 2021). Mechanistically, the antifungal activity of Fe_2O_3 NPs can be attributed to (i) ROS generation causing oxidative damage, (ii) cellular content leakage and disruption of membrane (iii) interaction with vital enzymes affecting respiration, and (iv) penetration into fungal cells enhancing stress and apoptosis (Ahmed *et al*., 2016). Thus, the combined results confirm that Fe_2O_3 NPs synthesized using *Vinca rosea* extract exhibit concentration-dependent antifungal activity through both structural disruption and oxidative stress mechanisms.

The biocontrol and PGP effects of Fe_2O_3 NPs were confirmed through water agar (fig 9 a,b) (Table 3), pot studies, and post pot trial enzyme assays. Fe_2O_3 NPs enhanced chickpea seed germination and vigor both under pathogen stress (FOC) and non-stress conditions, achieving up to 100% germination and maximum vigor index at 100 ppm, confirming their dual role in biocontrol and growth promotion, indicating the suppression of fungal infection and improved seedling vigor, consistent with Rathod *et* *al*. (2023). In table 4 *In vivo* pot trials showed that Fe_2O_3 NPs enhanced chickpea growth under both pathogen stress (FOC) and non-stress conditions. Treatments with 50–100 ppm improved shoot and root growth, leaf number, and biomass, indicating strong biocontrol and plant growth-promoting effects. Overall, Fe_2O_3 NPs demonstrated dual efficacy in disease suppression and growth promotion, confirmed statistically. Similar biocontrol and growth-promoting outcomes were reported in tomato plants exposed to ferric oxide NPs (Elbasuney *et al*., 2022), with the mechanism attributed to the activation of plant defense enzymes and improved nutrient uptake (Li *et al*., 2023). post pot trial enzyme assays supported these observations, SOD activity increased dose-dependently with nanoparticles up to 50 ppm compared to both the non-treated plant and FOC-treated plant figure 10 (a,b) , indicating a strengthened antioxidant defense against ROS and enhanced cell signaling and stress tolerance; however, at 100 ppm, activity slightly declined, likely due to toxicity or saturation effects. High proline indicates cellular stress response activation, In Figure 11 (a), the combination of FOC with increasing nanoparticle concentrations significantly elevated proline levels, indicating activation of stress-response pathways and improved stress resilienc (Hosseinifard *et al*., 2022). Similarly, in Figure 11 (b), nanoparticle treatments alone also induced proline accumulation in a dose-dependent manner, suggesting that the treatment triggers mild to moderate stress, thereby stimulating protective mechanisms such as osmotic balance, protein stabilization, and ROS scavenging. The phenolic content assay showed that nanoparticle and fungal treatments enhanced phenolic accumulation, which plays a vital role in antioxidant defense, pathogen resistance, and lignin synthesis for cell wall strengthening (Zhou *et al*., 2022 and by Kralova & Jampilek., 2021). Comparable findings were reported). where CuONPs induced significant phenolic accumulation in seedlings, supporting the role of nanoparticles in boosting plant defense responses. Chlorophyll and carotenoid assays revealed that nanoparticle treatments significantly enhanced photosynthetic pigments, which are essential for energy capture and plant development (Kreslavski *et al*., 2023). In Figures 13(a–d), both chlorophyll a and b levels increased notably at 100 ppm and 100 ppm + FOC compared to controls, indicating improved photosynthetic efficiency and reduced risk of chlorosis. Similarly, Figures 13 (e, f) showed a concentration-dependent rise in carotenoids, with maximum accumulation at higher nanoparticle levels, suggesting strengthened photoprotection and stress tolerance. Collectively, these results demonstrate that Fe_2O_3 NPs not only suppress *Fusarium* infection but also promote growth and enhance protection through antioxidant, osmoprotective, and structural defense mechanisms. Though present study shows positive effect but in long term nanoparticles may have toxic effect on the plants or soil microbes, for which further long term research can be done.

5. Conclusion

Fe_2O_3 Nps were successfully synthesized using *Vinca rosea* extract and characterized through UV–Vis, FTIR and SEM. The nanoparticles showed antifusaric activity against FOC, with reduced fungal growth, biomass, and structural damage observed under SEM. *Vinca rosea* derived nanoparticls also improved plant growth and defense mechanisms by enhancing germination percentage, vigor index, antioxidant enzyme (SOD) activity, proline and phenolic accumulation, as well as chlorophyll and carotenoid content, particularly at higher concentrations. These findings confirm the potential of Fe_2O_3 Nps in biocontrol and plant development, supporting their application as biofertilizers and biopesticides.

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6. *Azadirachta indica* (Neem) Tree And Its Botanical Characteristics

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Fig. 1: A neem tree

1. 1. Introduction

Azadirachta indica A. Juss., commonly known as the neem tree, is a tropical evergreen species (deciduous under arid conditions) belonging to the family Meliaceae. The botanical name derives from the Persian phrase *"Azad Dirakht-i-Hind"*, meaning "free tree of India", a reference to the species' natural resistance to pests and diseases and its ecological compatibility. Neem is indigenous to the Indian subcontinent and has long been recognized for its environmental resilience and medicinal value.

Neem has held a prominent position in traditional Indian medicine (Ayurveda) for over four millennia. In Sanskrit, it is referred to as *'Arishta'* (meaning "complete, perfect, and imperishable") and *'Nimba'*, derived from *'Nimbati Swasthyam Dadati'*, which translates to “that which bestows health.” The pharmacological attributes of neem are extensively documented in classical Ayurvedic texts such as the Charaka Samhita and Sushruta Samhita, foundational scriptures of Indian medicinal science.

Internationally, neem is also known as Indian lilac or Margosa, while the Persian name *“Azad Darakht-e-Hind”* further emphasizes its Indian origin and utility. Beyond its cultural and medicinal significance, neem is considered a key component of India’s plant genetic resources**.**

Neem is often described as one of the most studied and promising tree species of the 21st century, owing to its exceptional biological versatility. Among tropical trees, neem yields the highest number of valuable non-wood products, including leaves, bark, flowers, fruits, seeds, oil, gum, and neem cake. These products exhibit a broad spectrum of bioactive properties, making the species invaluable in multiple domains.
Phytochemical and pharmacological studies have demonstrated that neem-derived compounds possess numerous biological activities, such as:

- Antiallergenic
- Antidermatic
- Antifeedant
- Antifungal
- Anti-inflammatory
- Antipyorrhoeic
- Antiscabic
- Cardiotonic
- Diuretic
- Insecticidal
- Larvicidal
- Nematicidal
- Spermicidal

Owing to these diverse bioactivities, neem finds extensive applications in pharmaceuticals, agriculture (as a biopesticide and soil amendment), cosmetics, and environmental management, positioning it as a vital green resource with significant scientific and socio-economic relevance.

1. 2. Classification

Kingdom: Plantae

Division: Magnoliophyta

Order: Sapindales

Family: Meliaceae Juss.

Genus: *Azadirachta* A. Juss.

Species: *Azadirachta indica* A. Juss.

1. 3. Taxonomy And Nomenclature

Azadirachta indica A. Juss. Mem. Mus. Hist. Nat. 19: 221. 1830 (publ. 1831) *Antelaea azadirachta* (L.) Adelb. Blumea vi. 315. 1948. *Antelaea canescens* Cels ex Heynh., Alph. Aufz. Gew. 38. 1846. *Antelaea javanica* Gaertn. Fruct. Sem. Pl. i. 277. 1788. *Azadirachta indica* var. *minor* Valeton Pl. Bogor. Exs. 66. 1904. *Azadirachta indica* var. *siamensis* Valeton Pl. Bogor. Exs. 66. 1904. *Azadirachta indica* subsp. *vartakii* Kothari, Londhe & N. P. Singh Bull. Bot. Surv. India 39: 181. 1997 (publ. 2001). *Melia azadirachta* L. Sp. Pl. 385. 1753. *Melia fraxinifolia* Salisb. Prodr. Stirp. Chap. Allerton 317. 1796. *Melia indica* (A. Juss.) Brandis Forest Fl. N.W. India 67. 1874. *Melia parviflora* Moon Cat. Pl. Ceylon 35. 1824. *Melia pinnata* Stokes Bot. Mat. Med. 2: 482. 1812.

Type: Lectotype: Herb. Hermann 2: 56, No. 161 (BM-000594618), (See fig. 2).

Neem (*Azadirachta indica* A. Juss.) is belongs to family Meliaceae. The neem tree is described as *Azadirachta indica* by De Jussieu (1830) and belongs to order Rutales but recent classifications describes that belongs to order Sapindales (APG IV).

Fig 2: Type specimen of *Azadirachta indica* A. Juss

1. 4. Vernacular Names

Indian lilac, Margosa, Neem, Nim, Nimba. Limba, Limbo, Nimb, Verbu, Vepa, Veppam, etc.

1. 5. Botanical Characters

Small to medium-sized tree, usually evergreen, 15-30 m, with a round, large crown up to 20 m in diameter; branches spreading; bole branchlets for up to 7.5 m, up to 90 cm in diameter, sometimes fluted at base; bark moderately thick, with small, scattered tubercles, deeply fissured and flaking in old trees, dark grey outside and reddish inside, with colourless, sticky foetid sap. Leaves alternate, crowded near end of branches, imparipinnate, 20-40 cm, pulvinus at base, exstipulate, light green, with 2 pairs of glands at base, otherwise glabrous; petiole 2-7 cm, subglabrous; rachis channelled above; leaflets 8-19, subsessile, alternate proximally and more or less opposite distally, ovate to lanceolate, sometimes falcate, 3.5-10 x 1.2-4 cm, glossy, serrate; acuminate; base unequal. Inflorescence axillary, many-flowered thyrsus, up to 30 cm; bracts minute and caducous; flowers hermaphrodite or male on same tree (polygamous), actinomorphic, small, pentamerous, white or pale yellow, slightly sweet scented; bract minute, deciduous; calyx imbricate, sepals 5, broadly ovate and thin, 1.5 mm, puberulous inside; corolla imbricate, petals 5, 6 mm, free, spathulate, spreading, ciliolate inside. Staminal tube *ca.* 5 mm, puberulous, 10-striate, 10-toothed; teeth 2-lobed; anthers oblong, basifixed. Gynoecium tricarpellary, syncarpous. Ovary sub-globose, trilocular, 2 ovules in each locule although usually one and rarely two, mature into a seed, placentation axile at base and parietal towards upper portion of ovary; style linear, *c.* 2.5 mm; stigma trifid. Fruit 1 seeded drupe, ellipsoidal, 1-2 cm, greenish, greenish yellow or purple; exocarp thin, mesocarp pulpy, endocarp cartilaginous; seed ovoid or spherical; apex pointed; testa thin, composed of a shell and a kernel (sometimes 2 or 3). (See fig. 3)

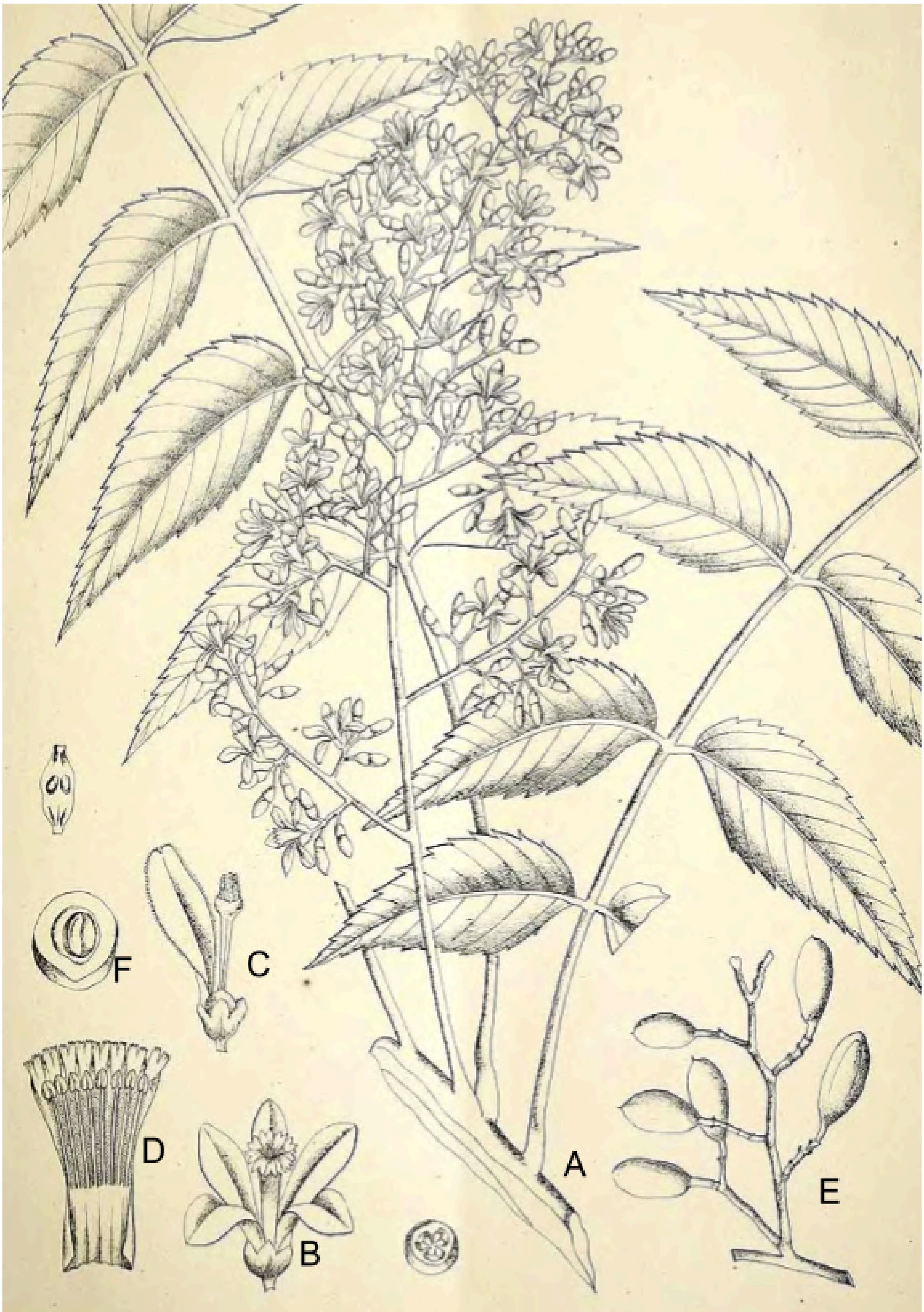

Fig.3: *Azadirachta indica* A. Juss. (A) A plant twig with flowers; (B) A flower; (C) Androecium; (D) Gynoecium; (E) Fruits; (F) T. S. of Ovary.

1. 6. Biology

Neem trees (*Azadirachta indica*) typically begin flowering and fruiting at approximately 4 to 5 years of age; however, commercially viable seed yields are generally not attained until the trees reach 10 to 12 years. Pollination is primarily entomophilous, with honeybees and other insects serving as the main pollinators. The observation that some isolated individuals fail to produce fruit suggests the presence of self-incompatibility mechanisms within the species.

The timing of flowering and fruiting is largely influenced by geographical location and climatic conditions. For example, in Thailand, neem is capable of flowering and fruiting year-round, while in East Africa, where seasonal variations are more pronounced, these phenological events are restricted to specific periods aligned with the regional climate. Following anthesis, the fruits typically mature within approximately 12 weeks. The ripe fruits are consumed by bats and birds, which play a crucial role in seed dispersal. Neem trees are long-lived, with individual specimens known to survive for over 200 years under favorable conditions.

1. 7. Biophysical Limits

Neem is adapted to a wide range of environmental conditions. It typically occurs at altitudes ranging from sea level up to 1,500 meters. The species thrives in areas with mean annual temperatures reaching up to 40 °C and is well-suited to regions receiving annual rainfall between 400 and 1,200 mm.

It can establish a diverse array of soil types, particularly those that are neutral to alkaline. However, it demonstrates superior performance compared to many other species on shallow, stony, or sandy soils, and in sites where a hard calcareous or clay pan lies close to the surface. The optimal soil pH for neem growth ranges between 6.2 and 7.0.

1. 8. Habitat And Ecological Adaptability

Azadirachta indica is widely regarded as highly adaptable, often described as capable of growing “almost anywhere” in the lowland tropics. It readily establishes itself in a range of ecosystems, including shrub lands, open woodlands, grasslands, floodplains, riparian zones, coastal regions, and various forms of disturbed natural vegetation. Despite this adaptability, the species does not typically form dense, gregarious stands under natural conditions.

In its native and introduced ranges, neem associates with different vegetation types. In India, it is commonly found in mixed forests, often growing alongside Acacia spp. and *Dalbergia sissoo*. In Indonesia, it has become naturalized in lowland monsoon forests, while in Africa, it occurs in both evergreen forests and dry deciduous forests.

Mature trees of *A. indica* exhibit a degree of frost tolerance, although seedlings are significantly more susceptible to cold temperatures. The species is intolerant of waterlogging and will not survive in poorly drained or saturated soils. Neem has a high light requirement, though young seedlings can tolerate moderate shade during their early developmental stages.

1. 9. Parasitic Plants

Species such as *Dendrophthoe falcata*, *Tapinanthus* spp., and *Loranthus* spp. (family Loranthaceae) have been documented as parasitic plants on *Azadirachta indica*. In India, it is traditionally believed that neem trees infested by these parasites produce seeds with a noticeably sweeter taste.

1. 10. Reproduction And Dispersal

Azadirachta indica primarily reproduces through seed propagation, with its seeds being dispersed by frugivorous birds and bats that consume the fruit. Although the species is light-demanding at maturity, its seedlings are capable of germinating and establishing under dense shade. Due to its multiple ecological and economic benefits, neem has been widely introduced to arid and semi-arid regions worldwide. It continues to be promoted and planted, even in areas where it has shown invasive tendencies.

1. 11. Similar Species

Azadirachta indica is occasionally misidentified as species of the closely related genus *Melia*. However, the two genera can be readily distinguished based on leaf and ovary morphology. Species of *Azadirachta* exhibit once-pinnate leaves characterized by the presence of two pairs of glands at the leaf base - one orbicular and one elongated - and possess a trilocular ovary. In contrast, *Melia* species typically have bi- or tri-pinnate leaves, a single pair of orbicular glands, and a multi-locular ovary, usually with 4 to 8 locules.

1. 12. Origin And Geographical Distribution

Two species of the genus *Azadirachta* have been identified:

1. *Azadirachta indica* A. Juss., native to the Indian subcontinent
2. *Azadirachta excelsa* Jack., which is geographically restricted to the Philippines and parts of Indonesia

Azadirachta indica occurs naturally as a wild tree in countries such as India, Bangladesh, Myanmar (Burma), Pakistan, Sri Lanka, Malaysia, Thailand, and Indonesia. It has been extensively cultivated and naturalized in many parts of the world due to its adaptability and economic importance.

At present, *A. indica* is successfully growing in approximately 72 countries across Asia, Africa, Australia, and the Americas (North, Central, and South). It is recognized both in its native range and as an exotic species in numerous other regions.

Native Range:

Azadirachta indica is native to parts of South and Southeast Asia, including India, Pakistan, Sri Lanka, Bangladesh, Myanmar, Thailand, Malaysia, Indonesia, and also Senegal.

Exotic Range:

Neem has been introduced and successfully established in over 70 countries across Africa, the Americas, Australia, and parts of Asia, including nations such as Brazil, Nigeria, Egypt, Australia, the United States, Kenya, Mexico, and China, among many others.

1. 13. Neem Products

Food:

The fruits of neem are consumed fresh, cooked, or processed into desserts and beverages similar to lemonade. In some regions, young twigs and flowers are occasionally eaten as vegetables.

Fodder:

Despite their bitterness, neem leaves are used as fodder during dry seasons. The fruits serve as a vital food source for various wildlife species, particularly birds and bats, which consume the pulp but do not digest the seeds.

Fuel:

Neem wood is an excellent source of fuel. It is commonly used as firewood, and charcoal produced from it is of high quality. In India, neem oil is also traditionally burned in lamps.

Timber:

Although *A. indica* belongs to the mahogany family and exhibits some desirable timber traits, the grain is coarse and difficult to polish. Nevertheless, the wood is used to make furniture such as wardrobes, bookcases, and closets, as well as packing cases, thanks to its natural insect-repellent properties. The main trunk is widely used for construction posts and fencing due to its termite resistance. Wood density ranges between 720–930 kg/m^3 at 12% moisture content.

Gum and Resin:

A protein-rich exudate can be obtained by wounding the bark, commonly known in Southeast Asia as "neem glue". It shows potential as a food additive and adhesive.

Tannins and Dye:

The bark of neem contains 12–14% tannins, which is comparable to conventional tannin sources, offering potential use in dyeing and leather processing.

Lipids and Oil:

Neem seed oil is produced on a large scale in Asia for use in soaps, cosmetics, pharmaceuticals, and other non-edible products. Oil content may reach up to 50% of the kernel weight. As of 1990, neem oil was valued at approximately USD 700 per ton.

Pesticidal Properties:

The most significant bioactive compound in neem is azadirachtin, primarily concentrated in the seeds, although also present in leaves and other tissues.

Azadirachtin acts as an insect repellent, antifeedant, and growth regulator, disrupting metamorphosis and reproduction in many pest species. It does not typically kill insects outright but modifies their behavior, making it effective against over 300 insect species, including:

- ➔ Coleoptera (beetles, weevils)
- ➔ Lepidoptera (moths, caterpillars)
- ➔ Orthoptera (grasshoppers)
- ➔ Diptera (flies)
- ➔ Isoptera (termites)
- ➔ Thysanoptera (thrips)
- ➔ And others

Neem formulations (e.g., Azadi, Margocide, Nimbecidine) are widely used in agriculture. Homemade neem tea—made by soaking crushed seeds in water—is a traditional pesticide, applied directly to crops. Crushed kernels may also be used as a dry pesticide, particularly against stem borers. However, the efficacy of homemade preparations varies depending on seed quality, handling, water quality, and environmental conditions. The active compounds degrade quickly, providing protection for only about one week post-application.

While neem-based pesticides are generally safe for pollinators like honeybees, they may exhibit toxicity to fish, aquatic life, and some beneficial insects. Proper disposal—e.g., exposure to heat or sunlight—is essential to deactivate residual compounds.

Medicinal Uses:

Neem exhibits a wide range of pharmacological properties:

- ➔ Antifungal: Effective against several fungi pathogenic to humans.
- ➔ Antibacterial: Active against strains such as *Salmonella typhosa* and *Staphylococcus aureus*.
- ➔ Therapeutic: Various parts of the plant have anthelmintic, antiseptic, antiperiodic, diuretic, and purgative properties. Neem is used traditionally to treat boils, ulcers, eye infections, skin diseases, rheumatism, hepatitis, leprosy, and ringworm.
- ➔ Antimalarial: Neem leaf infusions are used in the treatment of malaria.

- ➔ Oral health: Chewing neem twigs as toothbrushes is a widespread practice, and has been shown to help prevent periodontal disease.
- ➔ Contraceptive: Neem oil acts as a spermicide and has been developed into intravaginal contraceptives (e.g., *Sensal* in India).
- ➔ Topical use: Neem oil is traditionally applied to the skin in both humans and livestock to treat various skin conditions; however, it is not safe for internal consumption.

1. 14. Ecological and Agricultural Services of Neem

Erosion Control:

Azadirachta indica is well-suited for dune stabilization and erosion control, particularly in arid and semi-arid regions. Its deep, well-developed root system enables it to access nutrients and moisture from deeper soil layers, making it highly drought-tolerant and effective in soil conservation programs.

Shade and Shelter:

With its broad, dense crown, neem functions effectively as a shade tree and is commonly planted along roadsides, in villages, and urban landscapes throughout the tropics. Its low branching habit also makes it valuable as a windbreak, providing shelter for crops, livestock, and human settlements in exposed environments.

Soil Improvement:

Neem by-products contribute significantly to soil fertility. The neem cake - the residue left after oil extraction from seeds—is widely used in India as an organic fertilizer and soil amendment. It enhances the efficiency of nitrogen-based fertilizers by reducing nitrification rates and suppressing soil-borne pests, including nematodes, fungi, and insects**.** Additionally, neem leaves and twigs are often applied as mulch or green manure, enriching the soil with organic matter.

Intercropping:

Neem has shown promise in agroforestry systems, particularly when intercropped with pearl millet **(***Pennisetum glaucum***)**, where it has demonstrated beneficial effects on crop performance and land productivity in Indian farming systems.

1. 15. Neem Databases

The following table summarizes major Neem resources databases around the globe.

NO.	NEEM RESOURCES	WEB LINKS
1.	Neem Foundation	www.neemfoundation.org
2.	Neem Tree Farms	www.neemtreefarms.com
3.	Nature Neem	www.natureneem.com
4.	The Neem in the world	www.neem.fr
5.	Using Neem	www.usingneem.com

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7. A case of intestinal coccidiosis in a young female Rhode Island Red chicken

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Abstract

Poultry coccidiosis is one of the most invasive protozoan diseases caused by various species of *Eimeria.* It caused a huge economic burden on commercial poultry industry through high morbidity and mortality. In this study, a young emaciated female Rhode Island Red chick with marked dehydration was presented for necropsy with history of profuse bloody diarrhoea following sudden death. Gross examination revealed hemorrhagic spots with "Salt and Pepper" appearance in jejunum and inflated hemorrhagic caeca with typhlitis filled with bloody intestinal content. The impression smear of intestine revealed presence of coccidian oocyst. Histopathological examination demonstrated marked villous atrophy, haemorrhages, infiltration of inflammatory cells along with the presence of unusual stages of coccidian oocyte. The gross and histological findings revealed *Eimeria necatrix* in the jejunum and *Eimeria tenella* in the caeca as the causative agents.

Key words: *Coccidiosis, Bloody diarrhoea, Eimeria sp., Poultry*

Introduction

India ranks among the top five poultry-producing countries in world with ranking second in egg production and third in broiler meat production (BAHS Statistics 2023). It is facing huge economical and productional challenges due to silent invader "poultry coccidiosis" (Choi *et al*., 2021)." Poultry coccidiosis is a disease caused by intestinal protozoan parasite *Eimeria* of phylum *Apicomplexa* targeting intestinal epithelial cells (Peek & Landman, 2011). It has high morbidity and mortality rates. There are main seven pathogenic species of *Eimeria* affecting poultry birds namely *Eimeria necatrix, Eimeria tenella, Eimeria brunetti, Eimeria mitis, Eimeria acervulina, Eimeria maxima* and *Eimeria praecox* affecting various parts of intestine resulting into sub-clinical signs such as enteritis, bloody diarrhoea, emaciation, anaemia, and sudden death. It is transmitted through ingestion of oocyst contaminated feed and water (Thenmozhi *et al.,* 2014; Shivaramaiah *et al.,* 2014). The pathogenesis and severity of these diseases depend upon types of *Eimeria species* involved in it (Morris *et al.,* 2007). This document reports a complete diagnosed necropsy case of poultry coccidiosis providing all valuable information about clinical signs, pathological lesions associated with it.

Material and methods

Necropsy Examination: A young emaciated female Rhode Island Red chick with marked dehydration was presented for necropsy in the necropsy hall, Department of Veterinary Pathology with history of profuse bloody diarrhoea, anaemia, dehydration, and sudden death. The carcass was subjected to complete necropsy examination as per standard protocols. External examination included body condition, hydration status, nutritional status, mucous membrane, external lesions or presence of ectoparasite and natural orifice was done. Thorough internal examination of all organs was carried out by opening thoracic and abdominal cavity to carefully observe all gross pathological lesions along contents in intestine and other organs.

Sample collection: Representative samples (especially intestine) were collected and fixed in 10% Neutral buffered formalin. Moreover, impression smears were made from distinct parts of intestinal contents for further analysis.

Histopathology: Formalin fixed tissues were processed and paraffin embedded and 3µm tissue sections were cut and stained with routine hematoxylin and eosin (H&E) stain for microscopic interpretations.

Results

Gross pathological findings

Intestine especially jejunum and ileum revealed diffused pinpoint haemorrhages on intestine showing characteristic “salt and pepper” appearance suggestive of *Eimeria necatrix* along diffused purpuric to ecchymotic haemorrhages on serosa of caeca (Figure 1). The cut section of intestine showed presence of hemorrhagic intestinal content along haemorrhages on mucosa along catarrhal enteritis (Figure 2).

Figure 1. Gross lesions showing characteristic of salt & pepper appearance on serosa of mid intestinal area along with haemorrhagic typhilitis (caeca)

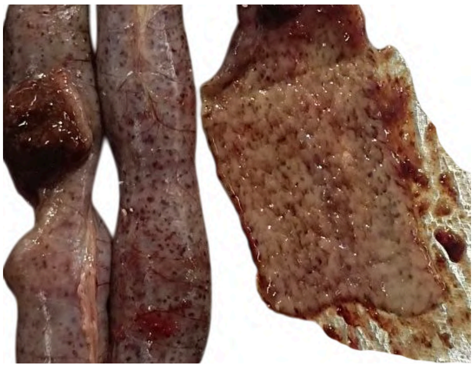

Figure 2. Gross lesion of the intestine showing haemorrhagic intestinal content along haemorrhagic mucosa and catarrhal inflammation.

Impressions smear examination:

The unstained impression smear of intestinal content revealed presence of unusual stages of coccidian oocyst. (Figure 3)

Histopathological findings

Histopathology of intestine revealed mucosal thickening, villous atrophy, haemorrhages and damaged epithelial cells with infiltration of inflammatory cells and presence of various stages of coccidian oocyte (merozoites-banana shaped, macrogamont-cartwheel like and microgamonts-dot like) (Figure 4-6) along with area of haemorrhages in the mucosa.

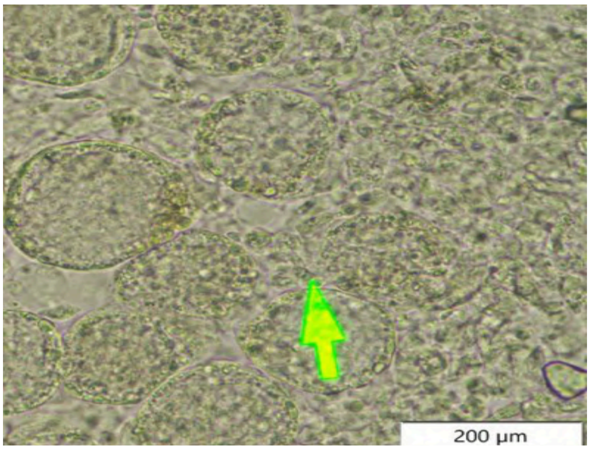

Figure 3: Impression smear showing presence of coccidian oocyst

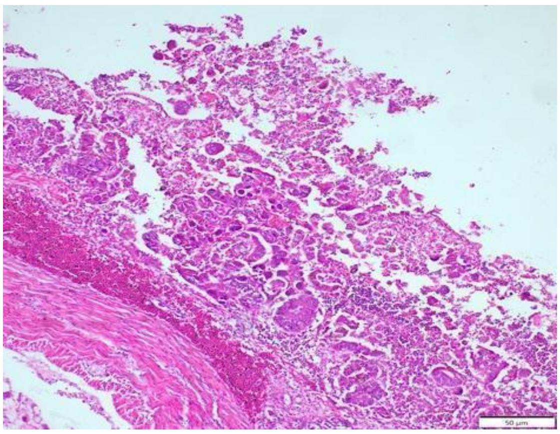

Figure 4: Microphotograph of the intestine showing atrophied villous, areas of haemorrhages, different developmental stages of coccidian

oocyst along inflammatory infiltration (H&E, 10X)

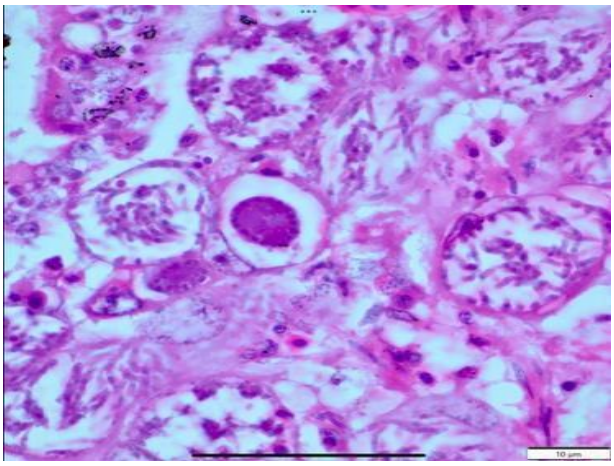

Figure 5: Higher magnification showing different developmental stages of coccidial oocyst -Meroziotes-banana shaped, macrogamont (cartwheel like), microgamonts (dot like) (H&E, 100X)

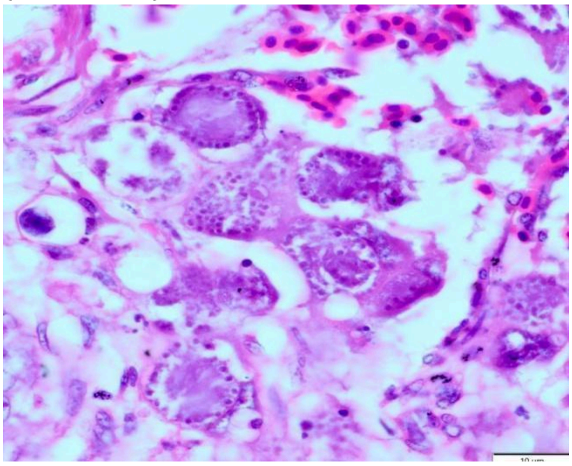

Figure 6: Higher magnification showing different developmental stages of coccidian along with hemorrhages (H&E, 100X)

Collectively, the gross, histopathological and impression smear findings were consistent with poultry coccidiosis caused by *Eimeria necatrix* and *Eimeria tenella (caecal coccidiosis).*

Discussion

In present case, gross necropsy revealed a haemorrhagic intestine with thickened mucosa and haemorrhagic typhlitis. These findings, along with impression smear and histopathology, confirmed a mixed infection with *E. necatrix* and *E. Tenella* (Maxwell & Johnson, 2017; Bafundo, 2025; McCullough, 2025). Co-infection of *Eimeria* species as observed in this study contributed to the pathogenicity of the disease (Haug *et al.,* 2008).

For prevention and control of poultry coccidiosis, strict biosecurity measures should be implemented so that disease introduction in the farm and its spread can be avoided. Biosecurity measures include restricted access to the farm, proper hygienic measures, appropriate ventilation, disinfection, and clean water usage. The litter should be maintained properly to avoid excessive moisture (wet litter) and thus prevent or reduce sporulation of oocyst. Further anti-coccidial drugs or coccidiostat such as monensin, sulphonamides, amprolium. Vaccination using non-attenuated or live attenuated vaccines can be employed (Klotz *et al*. 2005). Prophylaxis has been the ideal method to control poultry coccidiosis as therapeutics after the onset of clinical signs is often too late to prevent the outcome of the disease (Chapman 2009). Ahmed *et al* (2023) stated that besides vaccination, preventive anti-coccidial medications and proper handling methods on farms, the disease can be controlled through application of natural feed additives. It has been reported that resistance of the drugs also occurred which can be prevented through programs involving alternation amongst live parasite vaccination, chemoprophylaxis and/or poultry management (Blake *et al*. 2017).

Thus, in country like India where poultry coccidiosis is one of the highly prevent disease, prompt diagnosis and proper management along with prevalent treatment strategies (like anti coccidial drugs, vaccinations etc) should be implemented to control its extent.

Conclusion

This case report highlights the importance of correlated findings of various diagnostic approaches like necropsy, histopathology along impression smears to reveal presence of poultry coccidiosis. Early recognition of such lesions and prompt diagnosis are critical for flock-level disease management, implementation of control strategies, and reduction of economic losses in the poultry industry.

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8. Genetic Response Analysis of *Hordeum vulgare* under Tolerance and Toxic Thresholds of Sodium arsenate

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Abstract

Globally, human health and food security is under serious threat due to heavy metal contamination in the environment. Heavy metals like arsenic (As) is identified as the most hazardous because of its toxicity to plants and animals. This paper studies tolerance and toxicity threshold of arsenic in *Hordeum vulgare. Hordeum vulgare* ranks fourth cereal crop by quantity in the world and it is one of the healthiest grain. So its production increase will provide food to a large part of population. Sodium arsenate, ranging from 10^{-13} M to 10^{-1} M were used to treat seeds at varying concentrations. Morphological traits, particularly radicle length in seven-day-old seedlings from different accessions of *Hordeum vulgare*, were evaluated to determine the tolerance and toxic limits of As^{5+}. Results indicate that Sodium arsenate concentrations in the range of 10^{-3} M to 10^{-5} M showed no consistent effect on the root length of barley seedlings. However, at 10^{-4} M concentration shows the response appeared to be borderline, lying between tolerance and toxicity level among barley accessions.

Keywords: Heavy metal, *Hordeum vulgare,* Sodium arsenate, tolerance, genetic variability.

Introduction:

Most dangerous pollutants of the environment are heavy metals. Their accumulation in soil depends on natural processes (like Rock formation, Soil development) and human activities. Pollutants which contribute the most are miming, fossil fuel burning, urban waste disposal, sewer discharge, municipal dumping sites, soil runoff, industrial metal processing, boating and phosphate fertilizers. Heavy metals rising concentration in soil can be related to soil nature and farming practices (sludge use in agriculture fields). Major decline in crop yields and soil health happens if these contaminants levels rise above safe limits. As a consequence, heavy metal concentration becomes a serious driver of environmental pollution. Heavy metals enter food chain through bioaccumulation and biomagnification. This poses deadly health risks to humans like neurological, renal and carcinogenic effects.

Metals having density more than $5g/cm^3$ are known as Heavy Metals. There are 90 naturally occurring elements, out of which, 53 are classified as heavy metals. About 17 of these heavy metals can enter animal and plant cells depending upon their soluble and physiological conditions. Some heavy metals, such as zinc (Zn), nickel (Ni), copper (Cu), vanadium (V), cobalt (Co), tungsten (W), and chromium (Cr), are generally harmless at low concentrations and may even be useful in trace amounts. Others, including arsenic (As), mercury (Hg), silver (Ag), antimony (Sb), cadmium (Cd), lead (Pb), and aluminum (Al), serve no nutritional purpose and are largely toxic to both plants and microorganisms.

Objective

Although we know a lot on heavy metal toxicity, little is known about the genetic variability in arsenic tolerance among barley accessions. In This study, three accession of barley (*Hordeum vulgare*) were examined to show that tolerance and toxicity levels of arsenate that may contribute to sustainable crop production in contaminated soils.

Material and Method

A series of experiments were done to identify a suitable concentration of sodium arsenate for studying genetic variability in barley (*Hordeum vulgare*) under As^{5+} stress. Thirteen different molar concentrations of sodium arsenate were tested on three barley accessions with three replicates per treatment. For each treatment, fifty seeds were placed in sterilized Petri dishes lined with cotton and layered between filter papers. The control group was grown in Hoagland's solution. Both control and treated sets were kept at 20 °C in complete darkness for seven days. After seven days, radicle lengths of the seedlings were measured. Mean radicle length was calculated for each accession and treatment concentration. The concentration at which radicle growth began to decline was considered the toxic limit, while the highest concentration supporting normal growth was regarded as the tolerance limit. From these trials, 10^{-3} M sodium arsenate was chosen as the working concentration for analyzing genetic variability in tolerance. To measure the impact of As^{5+}, a parameter known as the Response Coefficient (RC) was used, calculated with the formula:

RC = VT – VC / RC

Where ***VT*** is the value for the treated set and ***VC*** is the value for the control set. Negative RC values indicated inhibition, while positive values reflected stimulation.

Observations and Result

At low concentrations (10^{-13} to 10^{-9} M), radicle growth was comparable to the control, indicating no toxic effect. Sodium arsenate concentrations in the range of 10^{-3} M to 10^{-5} M showed no consistent effect on the root length of barley seedlings. However, at 10^{-4} M, the response appeared to be borderline, lying between tolerance and toxicity levels. A concentration of 10^{-3} showed a sudden reduction while 10^{-2} and 10^{-1} were deadly for seedlings. Considering the toxic effects observed, a concentration of 10^{-3} M sodium arsenate was chosen for treating the different accessions in order to analyze genetic variability in *Hordeum vulgare* (Table 1, 2 & 3).

Table-1

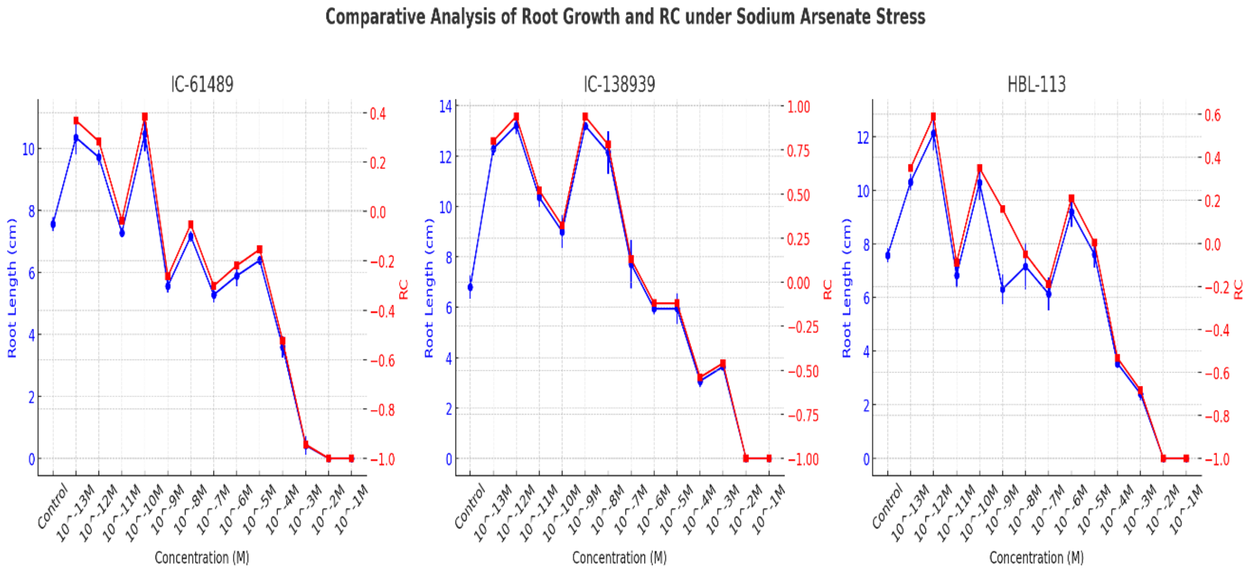
Accession	Treatment	Mean	± SE	Range	RC
IC-61489	Control	7.56	0.22	4-9.50	
	10^{-13} M	10.35	0.54	4.0-13.70	0.369
	10^{-12}M	9.71	0.24	3.8-14.5	0.284
	10^{-11} M	7.28	0.15	4.5-10.5	-0.036
	10^{-10}M	10.47	0.56	6.6-13.0	0.385
	10^{-9}M	5.56	0.21	4.0-7.80	-0.263
	10^{-8} M	7.16	0.16	3.6-10.0	-0.052
	10^{-7} M	5.28	0.26	3.0-7.00	-0.301
	10^{-6} M	5.89	0.35	4.0-8.50	-0.219
	10^{-5} M	6.39	0.15	3.5-10.8	-1.535
	10^{-4} M	3.59	0.35	2.0-6.50	-0.524
	10^{-3} M	0.42	0.31	0.0-1.80	-0.944
	10^{-2} M	0.00	0.00	0.0-0.00	-1.00
	10^{-1} M	0.00	0.00	0.0-0.00	-1.00

Table-2

Accession	Treatment	Mean	± SE	Range	RC
IC 138939	Control	6.80	0.46	4.30-6.30	
	10^{-13} M	12.29	0.25	8.20-9.80	0.80
	10^{-12}M	13.22	0.35	7.00-9.10	0.94
	10^{-11} M	10.36	0.38	9.80-11.90	8.5
	10^{-10}M	9.00	0.65	7.00-8.60	0.32
	10^{-9}M	13.2	0.16	8.40-10.30	0.94
	10^{-8} M	12.14	0.85	8.10-10.00	0.78
	10^{-7} M	7.71	0.96	8.00-9.40	0.13
	10^{-6} M	5.94	0.23	8.30-9.80	-0.12
	10^{-5} M	5.94	0.61	9.00-11.80	-0.12
	10^{-4} M	3.07	0.25	4.60-6.80	-0.54
	10^{-3} M	3.64	0.15	4.90-6.80	-0.46
	10^{-2} M	0.00	0.00	0.0-0.00	-1.00
	10^{-1} M	0.00	0.00	0.0-0.00	-1.00

Table-3

Accession	Treatment	Mean	± SE	Range	RC
HBL-113	Control	7.58	0.26	6.20-9.50	9.50
	10^{-13} M	10.31	0.31	8.00-12.60	0.35
	10^{-12}M	12.12	0.63	7.80-15.50	0.59
	10^{-11} M	6.83	0.45	5.80-8.80	0.09
	10^{-10}M	10.28	0.64	5.80-13.0	0.35
	10^{-9}M	6.31	0.56	5.00-7.80	0.16
	10^{-8} M	7.16	0.86	5.40-8.60	-0.05
	10^{-7} M	6.14	6.14	4.50-7.50	-0.19
	10^{-6} M	9.19	0.56	6.10-11.0	0.21
	10^{-5} M	7.61	0.49	5.80-10.0	0.003
	10^{-4} M	3.54	0.06	2.20-4.50	0.533
	10^{-3} M	2.41	0.25	2.00-3.00	-0.683
	10^{-2} M	0.00 0	0.00	0.00-0.00	-1.00
	10^{-1} M	0.00	0.00	0.00-0.00	-1.00



Discussion

Guidelines for determining the tolerance and toxicity limits of heavy metals has given by Berry (1986). In this study, Roots are the first organ to come in contact with arsenic, so RC value of root length was considered as most reliable parameter for estimating tolerance. RCs were calculated for radicle length across thirteen molar concentrations of sodium arsenate (10^{-13} M–10^{-1} M) used to treat different accessions of *Hordeum vulgare*. Within the tolerance range (10^{-13} M–10^{-5} M), radicle growth remained largely unaffected. However, at the borderline concentration of 10^{-4} M, root length began to decline, and beyond this point, it decreased sharply with increasing concentrations. Pronounced toxicity was imposed by 10^{-2} M while 10^{-1} M was lethal for all cultivars. Berry *et al*. (1986) also reported similar findings, he studied copper and cobalt tolerance in three closely related taxa of *Silene* L. from Zaire, where yield and metal uptake showed a comparable relationship. The present results clearly indicate that the degree of toxicity exerted by As^{5+} depends not only on its concentration but also on the genetic makeup of the accessions under study. Inhibition of root length caused by heavy metals may be due to metal interference with cell division, together with inducement of chromosomal aberrations and irregular mitosis are reported by (Jiang *et al* 2001, Liu, *et al* 2003). In a study of chromite polluted soil in five mung bean verities , noted that root growth was significantly affected 28th days after root emergence because seedling are more sensitive than seed germination for measurement of the toxic effect of chromium pollution. (Samantaray *et al*. 1999). Arsenate toxicity can interfere with ATP production, disrupt phosphate metabolism, and generate reactive oxygen species (Meharg & Hartley-Whitaker, 2002; Finnegan & Chen, 2012),

One of the earliest and most evident toxic effects of heavy metals is the inhibition of root growth in plants grown in a simple nutrient medium containing the test metal. This approach assumes that as metal concentration increases, root growth of tolerant plants will be inhibited to a lesser extent than that of sensitive plants Wilkins (1978) and Baker, A.J.M. (1987).

Conclusion

This study demonstrates that concentration-dependent phytotoxic effects are observed on barley seedlings exerted by sodium arsenate. Toxic limit of 10^{-3} M, being identified for most accessions, which can be taken as threshold for future tolerance screening. Variability among accessions indicates the potential for selecting arsenic-tolerant genotypes, which can be used for cultivation in contaminated areas or further breeding programs. A lot of studies demonstrated that low arsenic concentration stimulate plant growth while excessive harm plants.(Han *et al*., 2002; Abedin & Meharg, 2002; Abedin *et al*., 2002; Carbonell-Barrachina *et al*., 1998; Rahman *et al*., 2007; Wang *et al*., 2002). Further studies involving parameters like, Biochemical analyses, cytological analyses and yield patterns would help us in screening of relatively resistant cultivars.

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9. Isolation and Characterization of *Bacillus subtilis* as a Multifunctional Plant Growth-Promoting Rhizobacteria (PGPR) for Sustainable Agriculture

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Abstract

The escalating global population and increasing demand for food production have led to an excessive reliance on synthetic agrochemicals, which pose significant environmental and public health risks. This study explores a sustainable alternative by isolating and characterizing a potent Plant Growth-Promoting Rhizobacteria (PGPR) from the rhizospheric soil of the Gulf of Khambhat, Gujarat, India. From an initial pool of twelve isolates, a strain identified as *Bacillus subtilis* HP 12 was selected as the most promising candidate due to its multifunctional traits. Strain produced 8.0 µg/ml and 53 µg/ml of indol-3-acetic acid (IAA) and solubilized phosphate, respectively, confirmed by quantitative analysis. Moreover, the isolate's also displayed capabilities for nitrogen fixation and the solubilization of both potassium and zinc. The strain did not exhibit the production of ammonia or hydrogen cyanide (HCN). The practical efficacy of a talc-based bioformulation of *Bacillus subtilis* HP 12 was validated through pot trials on chickpea (*Cicer arietinum*). Seeds treated with the bioformulation showed marked improvements in key vegetative parameters, including root length, shoot length, and number of leaves, compared to untreated controls. The findings confirm that the isolated *Bacillus subtilis* HP 12 strain possesses multiple, complementary growth-promoting attributes, positioning it as a strong candidate for an effective, eco-friendly biofertilizer. The study underscores the potential of PGPR, particularly spore-forming species like *B. subtilis*, to reduce dependence on chemical inputs and contribute to improved crop yield and long-term soil health.

Keywords: *PGPR, Bacillus subtilis, IAA, Phosphate solubilization, Nitrogen fixation, Biofertilizer, Sustainable Agriculture*

Introduction

Worldwide agricultural productivity is depending on the highly intensive use of synthetic fertilizers and pesticides to cater the ever-growing food demands of a growing human population. However, intensive chemical-dependent farming has created a multitude of long-term challenges for environmental sustainability and human well-being (Linhart *et al*., 2019; Rathod *et al*., 2023b). The overuse of chemical fertilizers can lead to significant soil degradation, disrupting the delicate balance of beneficial microorganisms that are essential for nutrient cycling and overall soil fertility (Kenneth *et al*., 2019; Khan, Bano, Rahman, *et al*., 2019). Additionally, these agrochemicals can be spread from agricultural fields via surface runoff, to the contaminate rivers, lakes, and oceans which can in turn disrupt aquatic ecosystems and threatening biodiversity at multiple trophic levels (Banwo *et al*., 2021; Gani *et al*., 2021).

This over reliance on chemicals has its consequences such as development of pesticide resistance in pest demands either increase dosage or produce more toxic pesticides to maintain efficacy (Abdelkader *et al*., 2022). This self-perpetuating problem increases economic burden on farmer and also escalate environment damage and public health risk. Majority of these pollutants are xenobiotic and prolong exposure to human cause severe health related issues such as respiratory disorder and cancers. Therefore, it is an imperative need to hinge toward sustainable agricultural practices that maintain soil health with minimum damage to environment for ensuring the long term food security with maintaining ecological integrity.

To mitigate the challenges posed by conventional agriculture, biological alternatives garner significant attention. The group of soil inhabiting microorganisms that populate the root systems of plants termed as Plant Growth-Promoting Rhizobacteria (PGPR) bid a green, eco-friendly solution to enhance crop productivity and health (D. Goswami *et al*., 2017; Luziatelli *et al*., 2024; Rani *et al*., 2019). The rhizosphere is a dynamic centre of robust biological and chemical interactions represents the narrow zone of soil surrounding plant roots. Plants release a wide variety of root exudates, which act as chemoattractant for beneficial bacteria, creating a unique and favourable territory for

PGPRs (K. B. Patel & Thakker, 2020).

PGPRs promote plant growth through multifaceted mechanisms which can be categorized into two major groups, direct and indirect mechanisms. Direct mechanisms include improving nutrient availability and modulating plant hormone levels (Kenneth *et al*., 2019; Rathod *et al*., 2024). For example, many PGPRs are known to execute biological nitrogen fixation, converting atmospheric nitrogen (N_2) into a directly assimilable form like ammonia (NH_3) for plants (Kaschuk *et al*., 2022). They also expedite the solubilization of precious nutrients that are often present as unobtainable form in the soil, such as insoluble phosphates, potassium, and zinc, by secreting organic acids and other chelating compounds. Furthermore, PGPRs produce and release phytohormones like auxins (e.g., indole-3-acetic acid or IAA), gibberellins, and cytokinins, which directly encourage root development, nutrient uptake, and overall plant growth (Sun *et al*., 2024; Yasmin *et al*., 2021; Zaidi & Khan, 2017). PGPRs can protect plants from pathogens through indirect mechanisms, by producing antimicrobial compounds, competing for nutrients and space, or inducing systemic resistance within the host plant (Blake *et al*., 2021; Dweipayan Goswami, Patel, *et al*., 2015).

Among the diverse genera of reported PGPRs, such as *Pseudomonas*, *Azospirillum*, and *Azotobacter*, the genus *Bacillus* stands out as a particularly important and widely reported group for agricultural applications (Gani *et al*., 2021). *Bacillus subtilis*, a Gram-positive, rod-shaped bacterium, is well-known for its metabolic flexibility and robust nature (Rathod *et al*., 2024). A key biological trait that differentiates *Bacillus subtilis* from many other PGPRs is its ability to form heat- and desiccation-resistant endospores (Kloepper *et al*., 2004). This spore-forming capability provides an unequalled gain for the long-term viability of biofertilizer products, confirming that the microbial population remains effective during storage, transport, and application in challenging environmental conditions (Baliyan *et al*., 2022; D. Goswami *et al*., 2013). Existing literature supports that *B. subtilis* owns a wide range of beneficial traits, including nitrogen fixation, phosphate solubilization, and the production of phytohormones and other enzymes that enhance plant growth (Baliyan *et al*., 2022; Khan, Bano, & Babar, 2019).

Even with the documented benefits of PGPRs, their effective incorporation into commercial agriculture necessitates the isolation and rigorous characterization of strains with proven, multifunctional efficacy from different challenging environments. This study was undertaken with the objective of identifying and evaluating a potent PGPR from the distinctive ecological niche of the Gulf of Khambhat, Gujarat. The specific aims were threefold: first, to isolate and screen a diverse collection of bacterial strains for a wide array of plant growth-promoting traits; second, to perform a comprehensive *in vitro* characterization of the most promising isolate, including a detailed quantitative analysis of its key functional attributes; and third, to validate its efficacy through a controlled *in vivo* pot trial on an economically significant legume, chickpea (*Cicer arietinum*), to assess its potential as a sustainable biofertilizer.

Materials and Methods

Isolation and Morphological Characterization of Rhizospheric Bacteria

Rhizospheric soil samples were collected from the Gulf of Khambhat, Gujarat, and used for the isolation of bacterial microbiota. A small amount of soil was suspended in sterile distilled water and serially diluted. The diluted suspension was then spread onto sterile nutrient agar plates. The plates were incubated at 37 °C for 48 hours to allow for bacterial growth. After incubation, twelve discrete pure culture isolates were obtained based on disparities in colony morphology, such as shape, size, color, elevation, margin, and surface. The purity of the isolates was ensured by repeatedly streaking on fresh nutrient agar plates. The purified cultures were maintained on nutrient agar slants and stored at 4°C for further study. Based on the initial screening of PGPR properties, the selected isolate was characterized using biochemical and physiological properties, (Khan, Bano, & Babar, 2019; Khan, Bano, Rahman, *et al*., 2019).

Screening for Plant Growth-Promoting Traits

The twelve isolated strains were screened for a series of plant growth-promoting traits using conventional protocols. The strain exhibiting the most promising multi-trait profile was selected for quantitative analysis and subsequent pot trials.

Indole-3-Acetic Acid (IAA) Production: Quantitative and Qualitative Assessment

The ability of the isolates to produce IAA was evaluated using the Salkowski's reagent method (K. B. Patel & Thakker, 2019). Each isolate was inoculated into nutrient broth

(NB) augmented with 200 µg/ml of L-tryptophan and incubated for five days at 37 °C. After incubation, the cultures were centrifuged at a 6000 rpm for 5 mins to pellet the bacterial cells. The equal proportions collected supernatant and Salkowski's reagent were mixed. The production of IAA was indicated by development of pink color. The quantity of IAA produced was measured by determining the absorbance of the solution at 530 nm using a Systronics-117 UV-Visible spectrophotometer against a standard curve prepared using standard IAA concentrations (10 to 100 µg/ml).

Phosphate Solubilization Assay: Quantitative and Qualitative

Phosphate solubilizing ability was qualitatively screened by inoculating the organisms on Pikovskaya's agar and incubating them at 37 °C for five days. The development of a clear zone around a colony showed the solubilization of insoluble phosphate (K. B. Patel & Thakker, 2020). The stannous chloride method based on the principle of reducing phosphomolybdic acid to a blue complex was employed for quantitative estimation of phosphate solubilized. The isolate was inoculated into 100 ml of Pikovskaya's broth and incubated at 30 °C for five days. A 1 ml culture aliquot was centrifuged, and 0.1 ml of the supernatant was diluted with 0.9 ml of distilled water. To this, 1 ml of chloromolybdic acid and 0.25 ml of chlorostannous acid were added and mixed. The final volume was made up to 5 ml with distilled water and absorbance was measured at 600 nm using a Systronics-117 UV-Visible spectrophotometer. The concentration of soluble phosphate was determined against the standard curve of Monobasic potassium phosphate (KH_2PO_4) (10 to 100 µg/ml).

Screening for Potassium and Zinc Solubilization

Qualitative screening for potassium solubilization, isolates were inoculated on Alaksandrov's agar, followed by incubation at 37 °C for five days (P. Patel *et al*., 2021). The presence of a clear zone around the colonies indicated the release of soluble potassium from insoluble form. Similarly, zinc solubilization was evaluated by inoculating the isolates on nutrient agar supplemented with 0.1% zinc oxide (ZnO) and incubated the plates at 27 °C for three days. A transparent clear zone around the colony was indicative of zinc solubilization (Yasmin *et al*., 2021).

Nitrogen Fixation Assay

To determine the isolates' ability to fix atmospheric nitrogen, the isolates were inoculated and grown on nitrogen-free Jensen's media (Dweipayan Goswami, Parmar, *et al*., 2015). The medium's composition included sucrose, dipotassium phosphate (K_2HPO_4), magnesium sulphate ($MgSO_4$), sodium chloride (NaCl), ferrous sulphate ($FeSO_4$), sodium molybdate (Na_2MoO_4), calcium carbonate ($CaCO_3$), and agar. The plates were incubated at 27 °C for two days. The visible growth of a colony on nitrogen-limited medium was reflected a positive result for nitrogen fixation, representing the organism's ability to utilize and fix atmospheric nitrogen for its own metabolic needs.

Screening for Ammonia and Hydrogen Cyanide (HCN) Production

The production of ammonia was tested by inoculating cultures in peptone water broth and incubated for five days at 27 °C. After centrifugation, 1 ml of Nessler's reagent was added to 200 µl of the supernatant, followed by dilution with 8.5 ml of autoclaved distilled water. A brown color development was indicated a positive result for ammonia production (Dweipayan Goswami, Patel, *et al*., 2015). For HCN production, the isolates were streaked on nutrient agar plates augmented with 5% glycine. A filter paper strip impregnated with 2% sodium carbonate (prepared in 0.5% picric acid) was placed inside the lid of the petri dish, which was then sealed with parafilm. The plates were incubated at 27 °C for five days. A change in the impregnated filter paper's color from yellow to brown indicated the production of HCN (Dweipayan Goswami, Patel, *et al*., 2015).

Preparation of a Talc-Based Bioformulation

A talc-based bioformulation was prepared for the pot trials to evaluate the *in vivo* efficacy of the selected PGPR strain. The formulation consisted of talc powder as the primary carrier, supplemented with 15 g of calcium carbonate ($CaCO_3$) and 10 g of carboxymethyl cellulose (CMC) per kg of talc (Rathod *et al*., 2023a). The components were thoroughly mixed in a sterile metal tray and autoclaved. A coating slurry was prepared by mixing the autoclaved talc formulation with a suspension of *Bacillus subtilis* at a concentration of 10^6 cells/ml. The purpose of talc as a carrier is to provide a protective and nutritive environment for the microorganisms, assisting their storage and transport from the laboratory to the field. Calcium carbonate acts as a buffer to maintain a favorable pH, and CMC improves the formulation's adherence to seeds and guards the bacterial cells from desiccation.

In Vivo Efficacy: Pot Trials on Chickpea (Cicer arietinum)

The effectiveness of the *Bacillus subtilis* bioformulation was evaluated using pot trials on chickpea seeds of the hybrid desi type. Seeds were surface sterilized to remove surface contaminants before being coated with the bioformulation. Treatments were assigned as follows: **T1** (Control), consisting of untreated seeds, and **T2**, consisting of seeds coated with the *Bacillus subtilis* bioformulation. Ten seeds were sown per pot. The experiment was conducted in June, during which temperatures ranged from 29 °C to 32 °C. After 10 days of vegetative growth, key parameters were estimated and recorded for each treatment. These parameters included root length, shoot length, and the number of leaves.

Results and Discussion

Isolation and Initial Screening of Rhizospheric Isolates

A total of twelve pure bacterial isolates were successfully obtained from the rhizospheric soil samples of the Gulf of Khambhat (Fig 1). These isolates were selected based on their distinct colony morphologies, which were meticulously recorded at 48 hours of growth. Following initial screening for a wide range of plant growth-promoting traits, the isolate designated as *Bacillus subtilis* HP 12 was chosen for comprehensive characterization and pot trials.

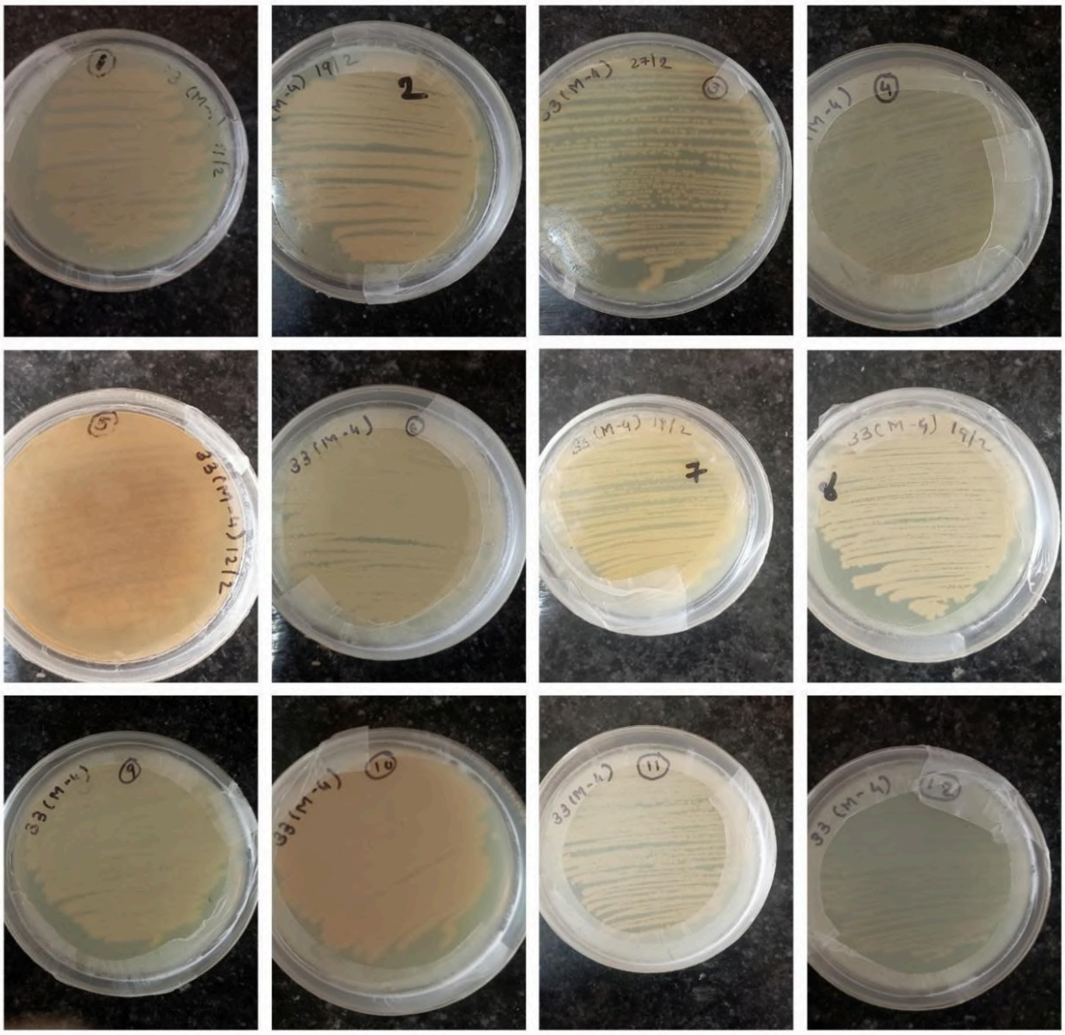

Fig 1. A total of 12 PGPR candidates isolated from the rhizospheric soil collected from gulf of Khambhat

The selection was based on its superior performance across multiple assays, demonstrating that its potential as a biofertilizer candidate was not predicated on a single trait but on a robust, multi-faceted profile. The ability to perform multiple complementary functions is crucial for a biofertilizer's efficacy in the complex and competitive soil environment. A comprehensive summary of its key biochemical and physiological traits is presented in Table 1.

Table 1: Biochemical and Physiological Characterization of *Bacillus subtilis* HP 12

	Bacillus subtilis
Gram staining	Positive
Motility	Motile
MR test	Negative
VP test	Positive
Citrate utilization	Positive
Urease	Negative
Gelatinase	Positive
Catalase	Positive
Oxidase	Positive
Sugar	Glucose - AG, Sucrose - A, Lactose - A, Xylose - negative, Maltose - AG, Mannitol - A
TSI	Slant - alkaline, Butt - acidic, H_2S - negative, Gas - positive

Multifunctional In Vitro Traits of Bacillus subtilis HP 12

The Efficacy of Auxin Production

Fig 2. Confirmation of IAA production using Salkowski's reagent, with color intensity correlated to standard IAA.

From the twelve isolates screened for indole-3-acetic acid (IAA) production, five showed positive results (Fig 2). IAA production was confirmed using Salkowski's reagent, with color intensity correlated to IAA concentration. The selected *Bacillus subtilis* HP 12 strain shown the highest IAA production, measuring 8.0 μg/ml after five days of incubation. The ability to produce IAA is significant because IAA is a key plant hormone that plays a crucial part in stimulating plant growth by improving root elongation, cell division, and differentiation (Khan, Bano, Rahman, *et al*., 2019). The increased root system under influence of IAA allowed the plant to spread through a larger soil volume, in so doing improving water and nutrient uptake (Şeker *et al*., 2017).

However, it is imperative to admit that the Salkowski's reagent method, while a widely adopted and convenient colorimetric technique, has acknowledged short-comings. The method may lead to an overestimation of the actual IAA concentration because it reacts with indole compounds rather than IAA alone (D. Goswami, Thakker, *et al*., 2015). Due to this, the reported value should be interpreted as a robust indicator of the isolate's potent auxin-producing capability rather than an exact measure.

Mechanisms of Nutrient Solubilization

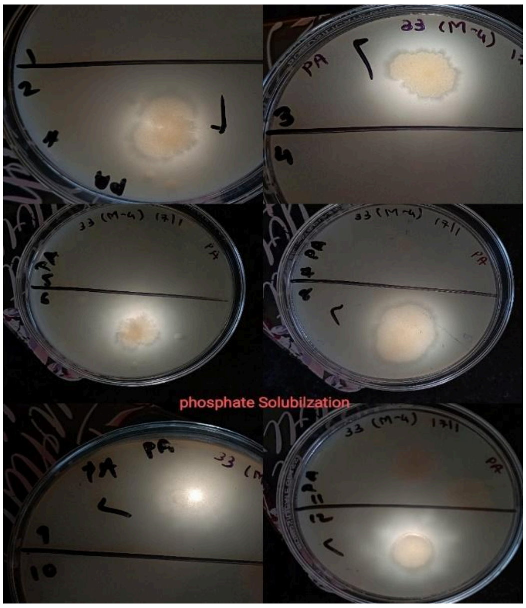

Fig 3. Confirmation of phosphate solubilizing ability of the isolates on Pikovskaya's agar with the zone of clearance surrounding colony indicated positive phosphate solubilizing trait.

Out of twelve isolates, five showed abilities to solubilise the phosphate on Pikovskaya's agar (Fig 3). The *Bacillus subtilis* HP 12 strain demonstrated remarkable nutrient solubilizing abilities. It effectively solubilized phosphate on Pikovskaya's agar, and quantitative analysis revealed a high solubilization rate of 53 μg/ml. The primary mechanism for microbial phosphate solubilizing ability is the secretion of organic acids, such as gluconic acid, formic acid, and citric acid, which decrease the pH of the surrounding medium (Goljanian-Tabrizi *et al*., 2016). This localized acidification converts insoluble mineral phosphates into soluble forms, making phosphorus readily available for plant uptake. This mechanism is crucial as a significant portion of phosphorus in the soil exists in an inert, fixed form that is inaccessible to plants.

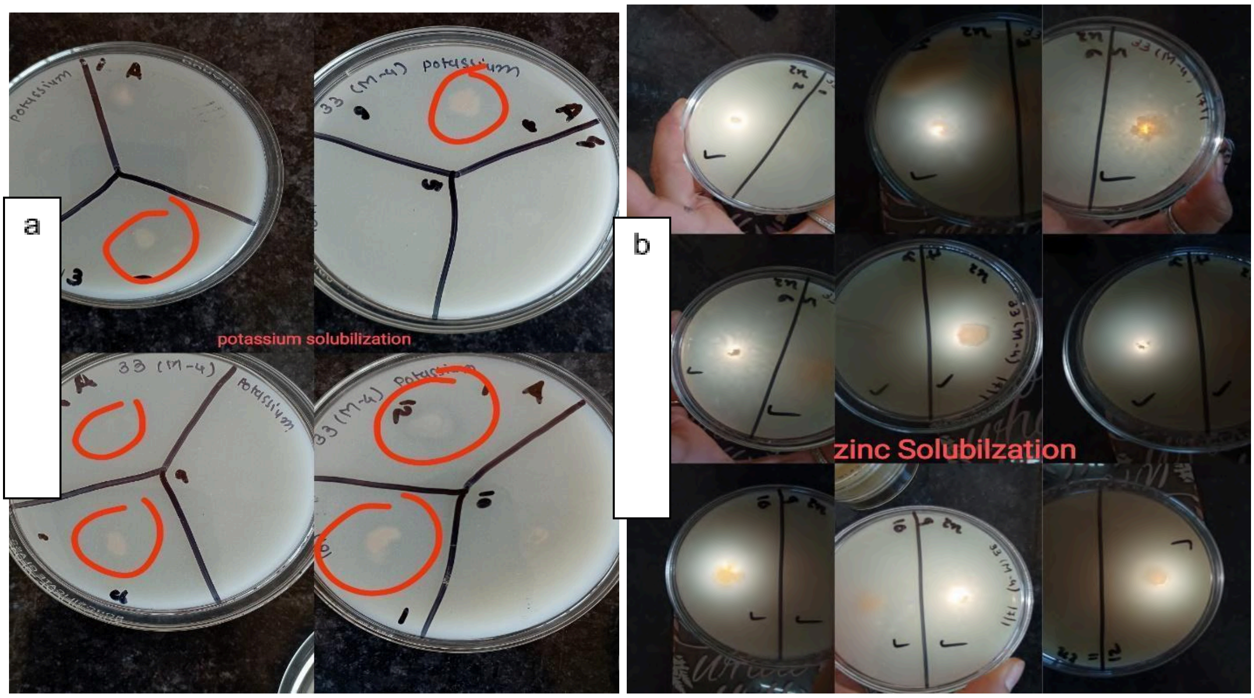

Fig 4. (a) Potassium solubilization activity of all the isolates Alaksadrove's Agar and (b) zinc solubilization on ZnO supplemented agar

Beyond phosphate, five isolates also exhibited the ability to solubilize potassium and zinc, as indicated by the clear zones on Alaksandrov's agar and nine isolates were shown zone of solubilization on nutrient agar supplemented with zinc oxide, respectively (Fig 4). Similar to phosphate solubilization, the release of organic acids is the major mechanism for the solubilization of potassium from minerals and zinc from insoluble compounds (Islam *et al*., 2018). The solubilization of these essential nutrients—potassium for ATP production and water uptake, and zinc for plant hormone synthesis and photosynthesis—is vital for overall plant health and productivity (Yasmin *et al*., 2021). Moreover, none of the isolates shown ammonia production as well as HCN production test positive.

The Importance of Nitrogen Fixation

In addition to its auxin production and nutrient solubilization capabilities, *Bacillus subtilis* HP 12 also demonstrated a positive nitrogen fixation ability, as evidenced by its growth on nitrogen-free Jensen's media (Fig 5). In total, 4 out of 12 isolates showed positive nitrogen fixation traits. Nitrogen fixation is a fundamental process in which certain bacteria, known as diazotrophs, convert inert atmospheric nitrogen (N_2) into a biologically usable form, primarily ammonia (NH_3), through the action of the nitrogenase enzyme complex (Şeker *et al*., 2017). This key capability allows the bacterium to stream a assimiable form of nitrogen to the host plant, thus reducing the plant's dependence on synthetic nitrogen fertilizers. The ability of the isolated strain to fix nitrogen represents a key step toward realizing a sustainable reduction in chemical fertilizer inputs (Kaschuk *et al*., 2022).

Fig. 5 Growth of the isolates tested on nitrogen free medium to confirm ability to fix atmospheric nitrogen

In Vivo Growth Promotion in Chickpea

Based on the different PGPR traits tested for all the twelve isolates, the isolate 12 owned all the traits excluding both production of ammonia and HCN. Therefore, the isolate 12 was subjected to identification based on the biochemical tests to be *Bacillus subtilis*. The efficacy of the *Bacillus subtilis* HP 12 bioformulation was verified on chickpea plants in a controlled pot trial. The results showed that the treated seeds reliably supported plants with enhanced vegetative parameters compared to the untreated control group, as also reported earlier (Rathod *et al*., 2024). The noticed enhancement in root and shoot lengths and the number of leaves in the treated plants associated with the multifunctional PGPR traits characterized in the laboratory assays. The improved root length can be credited to the ability of IAA production, which kindles cell elongation, while the overall increase in vegetative biomass could be a result of upgraded nutrient attainment from the soil due to the strain's ability to solubilize phosphate, potassium, and zinc, as well as fix atmospheric nitrogen (Khan, Bano, & Babar, 2019).

Table 2: Vegetative Parameters of Chickpea Plants Post Pot Trial

	Control	Treated
Root length (cm)	4.5±0.5	5.67±0.58
Shoot length (cm)	8.33±1.52	11.33±0.58
No of leaves	13.66±4.04	22.33±3.51

Fig 6. Comparative growth of (a) control and (b) treated chickpea seedlings

The significant change in plant in the measured parameters between the treated and control groups approves that the *Bacillus subtilis* HP 12 bioformulation is efficient plant growth promoter in a soil-based system.

Conclusion

This study isolated and characterized a multifunctional PGPR strain, *Bacillus subtilis* HP 12, from the rhizospheric soil of the Gulf of Khambhat. The isolate revealed a broad range of beneficial traits, including the production of the plant hormone IAA, solubilizing ability for phosphate, potassium and zinc. Most notably, its exhibited the ability to fix atmospheric nitrogen further augments its prospective as a wide-ranging biofertilizer. The positive *in vitro* results were directly corroborated by improvements observed in the vegetative growth parameters of chickpea plants in a controlled pot trial, asserting the strain's efficacy.

The isolated *Bacillus subtilis* HP 12 strain represents a promising, environmentally compatible alternative to synthetic agrochemicals. Its collective abilities to improve nutrient availability and promote plant growth hold significant potential to improve crop yield while also contributing to improved soil health with reduced environmental damage. The intrinsic capacity of *B. subtilis* to form endospores could provide a strategic advantage for the commercial development of a biofertilizer formulation, offering an improved shelf-life and greater reliability in diverse field conditions. However, future work should commence with large-scale, multi-site field trials to meticulously validate the strain's performance and stability under varied environmental and agronomic conditions. Simultaneously, efforts should be focused to optimizing the bioformulation to guarantee its long-term viability, addressing challenges such as stability during storage and scalability of production. This research serves as a critical step in the development of a biological-based solution for sustainable agriculture, paving the way for a more resilient and eco-friendly food production system.

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10. Sensitivity of Global tropospheric ozone and its relevance on the habitability of earth like planets

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Abstract

Tropospheric ozone, which absorbs UV radiation, plays a dual role in shaping planetary environments on Earth. In the layer near the Earth's surface, the troposphere ground-level "bad" ozone is an air pollutant that is a key ingredient of urban smog. But as we move higher in the stratosphere, "good" ozone protects life on Earth by absorbing some of the sun's UV rays. On Earth, variations in global tropospheric ozone are driven by complex interactions between natural processes and anthropogenic emissions, affecting air quality, radiative forcing, and ecosystem health. For Earth-like exoplanets, the sensitivity of tropospheric ozone becomes particularly relevant in assessing habitability, since elevated concentrations may pose risks to biological systems while also serving as a potential biosignature detectable in planetary spectra. This study revealed various assessments, integrating atmospheric chemistry and climate variation along with astrobiology to assess the major implications of tropospheric ozone for Earth-like planets.

Keyword: atmospheric ozone, stratosphere, Ozone hole, climate change

1. Introduction

As we know, protective ozone is present from the Earth's surface to about 12–15 km, and its formation leads to various complex chemical reactions occurring between the nitrogen oxides, volatile carbon compounds, and solar radiation. Literature study confirmed that various anthropogenic activities, such as natural emissions, large-scale atmospheric circulation, and cutting of trees, lead to alteration in tropospheric ozone. For Earth-like planets this major fluctuation of tropospheric ozone may serve as a critical indicator of atmospheric stability. Increased concentrations might affect living systems, both humans and animals, by impairing respiratory health, damaging green areas of earth, and at the same time downregulating climate feedbacks [2].

So, it is necessary to balance or bridge atmospheric science and astrobiology; several studies of tropospheric ozone provide an open sight into the delicate balance that governs planetary habitability [3].

Atmospheric ozone also plays a fundamental role in shaping the thermal structure of the atmosphere, yet its influence has often been overlooked in earlier assessments of how rising solar radiation affects Earth's climate. Recent studies demonstrate that ozone contributes to atmospheric warming by increasing both near-surface humidity and temperature. For the same solar irradiance, mean surface temperature is found to be about 7 K higher compared to conditions without ozone. Consequently, the moist greenhouse threshold—where water vapour becomes abundant in the stratosphere—is reached at lower solar irradiance (1572 W/m^2 versus 1647 W/m^2 in ozone-absent scenarios). These findings suggest that ozone lowers the upper solar irradiance limit at which Earth-like planets can sustain habitability [4].

The sensitivity of global tropospheric ozone thus plays a central role in regulating Earth's climate system and determining the potential habitability of Earth-like planets. Ozone in the lower atmosphere directly influences radiative balance, atmospheric chemistry, and the hydrological cycle through its interactions with water vapor and clouds. Further, it was found that variations in tropospheric ozone strongly affect surface temperature, stratosphere–troposphere exchange, and the moist greenhouse threshold. These processes determine whether a planet can sustain liquid water over long timescales, making ozone not just a marker of atmospheric evolution but also a key regulator of habitability. In comparisons with Earth's reanalysis data and modeling studies, they reinforce that the presence and distribution of ozone must be carefully considered when evaluating exoplanet atmospheres. Ultimately, ozone sensitivity emerges as a crucial diagnostic in assessing planetary environments, bridging the understanding of Earth's climate with the search for life beyond our planet [8].

2. Ozone depletion

The term "*ozone layer depletion*" is frequently used to refer to the stratospheric ozone layer thinning. The first signs of stratospheric ozone damage were discovered in 1974. Ozone depletion happens when the stratospheric ozone's natural production and destruction ratios are skewed in the direction of destruction. As a result, the earth would be directly exposed to the sun's heat and harmful ultraviolet rays [5,9].

2.1. The main steps of Ozone depletion are:

2.1.1. Emission of ozone-depleting substances (ODS):

Human activities release various ozone-depleting substances (ODS), including chlorofluorocarbons (CFCs), halons, carbon tetrachloride, methyl chloroform, and nitrous oxide, into the atmosphere. These compounds are chemically stable and can persist for long periods, gradually ascending into the stratosphere. As early as the 1970s, scientists proposed that human-derived CFC emissions were a primary driver of stratospheric ozone depletion.

When exposed to ultraviolet (UV) radiation, ODS molecules undergo photodissociation. CFCs, which have been extensively used as refrigerants in air conditioners and refrigerators, as cleaning solvents, blowing agents in foam production, and aerosol propellants, are especially stable in the stratosphere, with atmospheric lifetimes ranging from 50 to 100 years. In the upper stratosphere, UV radiation breaks these molecules apart, releasing chlorine (Cl) and bromine (Br) atoms. These highly reactive species readily interact with ozone, and a single chlorine or bromine atom can catalytically destroy thousands of ozone molecules [6,10].

2.1.2. Catalytic destruction of ozone:

Ozone is broken down by the chlorine atom, producing oxygen and chlorine monoxide (ClO). Another ozone molecule is broken down by the chlorine monoxide molecule, releasing an oxygen atom and a chlorine atom. Once again, the ozone molecule is broken down to oxygen by the free chlorine atom, allowing chlorine atoms to react with ozone. This indicates that the cycle is continued by the chlorine regeneration, which results in ongoing ozone depletion [11].

For example:

$$Cl + O_3 \rightarrow ClO + O_2$$
$$ClO + O_3 \rightarrow Cl + 2O_2$$

2.1.3. Polar stratospheric clouds (PSCs):

In the polar regions (especially Antarctica), very low temperatures form PSCs in winter. These clouds provide surfaces that accelerate ozone-destroying reactions. When sunlight returns in spring, large amounts of ozone are rapidly destroyed—forming the ozone hole.

An ozone hole refers to a significant thinning of the stratospheric ozone layer over Earth’s polar regions, especially Antarctica, during springtime. It is not an actual hole but an area where ozone concentrations drop to unusually low levels. This phenomenon occurs due to human-made chlorine and bromine compounds that become highly reactive under the extreme cold of the polar stratosphere.

The ozone layer protects the Earth's surface from dangerous ultraviolet (UV) radiation, so when it is lost, more UV rays can enter the atmosphere and reach the surface [6].

The Antarctic ozone hole continues to shrink thanks to international regulations like the Montreal Protocol and is expected to fully recover by mid-century. While the healing process is variable each year due to factors like weather patterns and occasional volcanic eruptions, the overall trend shows continued healing. Data from services like the European Environment Agency and Copernicus show that recent ozone holes, such as in 2024, have been smaller than those in the 2020-2022 period and are closing in line with historical averages.

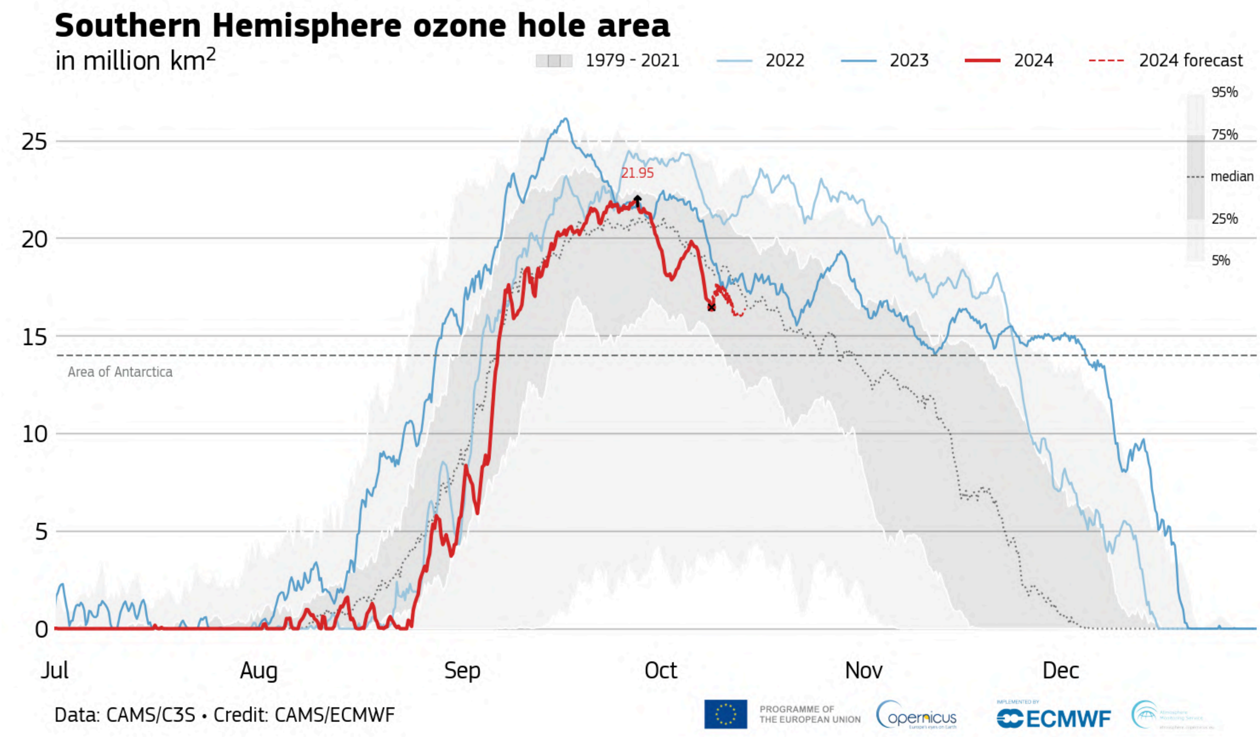

Figure 1. Southern Hemisphere ozone hole area

Here's a visual representation of the **maximum Antarctic ozone hole extent from 1979 to 2024**, drawn from satellite data. Let's explore the reasons behind its growth, peaks, and eventual gradual recovery [6].

2.2 Reason behind: Antarctic Ozone Hole Grow, and What Drives Its Year-to-Year Changes?

2.2.1. Human-Created Ozone-Depleting Substances (ODS)

During the latter half of the 20th century, extensive use of chemicals such as chlorofluorocarbons (CFCs), halons, and carbon tetrachloride resulted in the release of chlorine and bromine into the stratosphere, where they catalytically break down ozone molecules once activated. Although these compounds remain inert and stable in the lower atmosphere, they persist for long periods and become highly destructive in the stratosphere. Their accumulation over time played a major role in the severe ozone depletion observed above Antarctica (discoveringantarctica.org.uk, Ozone Secretariat, Earth Observatory).

2.2.2. Unique Antarctic Weather Conditions

Antarctica's stratosphere is extremely cold during its winter and early spring, reaching temperatures below −78 °C, which fosters the formation of Polar Stratospheric Clouds (PSCs). These clouds provide surfaces that accelerate ozone depletion chemistry by turning benign chlorine compounds into reactive radicals under sunlight (The Conversation, discoveringantarctica.org.uk, Climate.gov, Ozone Secretariat, Wikipedia) [12].

2.2.3. The Polar Vortex Effect

The strong and stable polar vortex isolates Antarctic air, trapping cold air and preventing mixing with ozone-rich air from lower latitudes. This allows ozone-depletion processes to continue unchecked until sunlight returns in early spring (The Conversation, theozonehole.org, DCCEEW, Ozone Secretariat).

2.3. Year-to-Year Variability: Dynamics Over Chemistry

Although ODS were the underlying cause, fluctuations in ozone hole size are largely driven by meteorological conditions:

- Colder, stronger vortex → larger ozone hole
- Warmer or weaker vortex → smaller ozone hole

Such variability stems from planetary wave activity and temperature changes in the stratosphere. Years with strong tropospheric wave activity result in warming of the lower stratosphere, thereby reducing ozone loss. Conversely, weaker wave activity leads to colder conditions—and more extensive depletion (atmosphere.copernicus.eu, giss.nasa.gov, World Meteorological Organization, Earth Observatory, theozonehole.org).

3. Beginning of Recovery Thanks to Global Action

Following the 1987 Montreal Protocol, emissions of the most damaging ODS declined sharply, slowing the expansion of the ozone hole by around 2000 (The Conversation, atmosphere.copernicus.eu, Earth Observatory, WIRED, DCCEEW).

Even now, meteorological variability continues to influence annual ozone hole size. The ozone layer is on a slow path to recovery, with projections estimating a return to pre-1980 levels over Antarctica by the mid-2060s (atmosphere.copernicus.eu, DCCEEW, Earth Observatory, WIRED).

Summary Table

Factor	Impact on Ozone Hole Extent
Ozone-depleting substances	Cause the formation and growth of the hole (CFCs, halons, etc.)
PSCs and cold stratosphere	Enable catalytic destruction of ozone via reactive chlorine
Polar vortex strength	Strong and cold vortex leads to bigger, deeper holes
Tropospheric wave activity	Affects stratospheric temperatures and thus ozone loss
Montreal Protocol	Banned key ODS, enabling gradual repair of the ozone layer

In a Nutshell

- Primary driver: Chemical destruction from ODS under the extreme Antarctic Spring conditions.
- Year-to-year changes: Largely dictated by atmospheric dynamics—temperature shifts and vortex behavior.
- Signs of recovery: Evident since ~2000 due to global policy actions like the Montreal Protocol, but full recovery will take decades.

4. Effect of ozone depletion

The primary possible consequence of ozone depletion is an increase in UV-B radiation at ground level. UV radiation rises by 2% for every 1% drop in ozone. Human health is anticipated to suffer greatly from increased exposure to solar UV-B radiation, which raises the risk of skin cancer, eye conditions, and infectious infections. UV radiation can damage the eye's cornea and lens, causing corneal cataracts, which can cause blindness. It can negatively affect the immune system, increasing the incidence of non-melanoma skin malignancies in light-skinned individuals and causing a range of infectious infections. Ozone compounds can cause respiratory issues, chest pain, throat inflammation, and lung function impairment [6].

4.1. Other effects of reduced ozone include:

4.1.1. Reduced plant growth is one of the effects of increased UV radiation, which can also change the timing and quantity of flowering.

4.1.2. Genetic alterations: a higher chance of mutation can lead to the emergence of small organisms.

4.1.3. Property damage: Materials that are susceptible to ultraviolet radiation may deteriorate or age more quickly.

4.1.4. Effects on marine ecosystems: Ultraviolet radiation can impact the survival of microscopic marine organisms such as plankton, which play a vital role in converting atmospheric carbon dioxide into oxygen, by disrupting their orientation and mobility. Such disturbances can cascade through and destabilize the entire ecosystem.

A reduction in stratospheric ozone may also result in elevated ozone concentrations in the lower atmosphere. At ground level, ozone is regarded as both a pollutant and a greenhouse gas, contributing to climate change and global warming. However, one potential benefit of this shift is the enhanced ability of sunlight to stimulate vitamin D production in humans, representing a notable positive outcome of ozone layer depletion.

5. International Agreements & Policies

- Montreal Protocol (1987): The most successful global treaty, which phased out production and use of ozone-depleting substances (ODS) like CFCs, halons, and carbon tetrachloride.
- Kigali Amendment (2016): Extended the protocol to reduce hydrofluorocarbons (HFCs)—though not ozone-depleting, they are potent greenhouse gases.
- Continued global cooperation is essential to prevent illegal production of ODS.
- Reduction of Ozone-Depleting Substances
- Ban/phase out CFCs, halons, methyl bromide, and other ODS.
- Adopt alternatives: Use hydrofluoroolefins (HFOs), hydrocarbons, and other environmentally safer refrigerants/propellants.
- Encourage industries to shift to ozone-safe technologies for refrigeration, air-conditioning, and foams.

6. Environmental & Technological Measures

- Promote ozone-friendly products labeled as “CFC-free.”
- Develop greener agricultural practices to reduce methyl bromide use (soil fumigant).
- Improve waste management to safely dispose of old refrigerators, ACs, and aerosols containing ODS.

7. Awareness & Education

- Public campaigns to reduce use of ODS-containing products.
- Encourage sustainable consumer behavior and support eco-friendly industries.
- Strengthen monitoring systems to detect illegal ODS emissions.

8. Individual Actions

- Choose energy-efficient, ozone-safe appliances.
- Properly service and recycle refrigerators/ACs to avoid ODS leakage.
- Support policies and brands that prioritize ozone protection.
- Reduce nitrous oxide emissions (from fertilizers and fossil fuels), which are now a major ozone-depleting gas.

In summary: Ozone depletion can be controlled mainly by phasing out ozone-depleting chemicals, enforcing international agreements like the Montreal Protocol, switching to eco-friendly alternatives, and raising awareness at both global and local levels.

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11. Role of Peer Review in Paper Writing in Scientific Journals and It's Importance

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Abstract

'Peer Review' simply refers to judging the quality and content level of a manuscript for publication in a Scientific Journal as a Research Paper. It is usually done by an individual having an expertise or specialization in any field. Peer reviewing helps in providing fair and candid comments and opinions related to the Correctness of the script, it's appropriateness of the content that truly serves the aim and objectives of the Journal in which it has to be published and it also fulfills the purpose of originality and uniqueness in an article by discouraging Plagiarism. It is based on the idea that only true peers or true friends can show one the right path. It checks the relevance, significance of the research and correctness of the methodology of an experiment, elevating the quality of the Journal. Prerequisites for Peer Reviewing include giving comments and reviews on time, suggesting ways and methods to improvise on the deficiencies and lacunae in the manuscript. A paper, rejected once, can be submitted again but to another Journal after revisions or after a time gap.

Key Words - Peer, Review, Reviewer, Journals, Paper, Publication

A "Peer" is defined as a " a person of equal standing". Scientific recognition or reputation and respect among others are intimately associated with Peer Opinion. Peer reviews are used in

1. Selecting an Employee, determining the Promotions.
2. Award or Funding or Grant for a particular cause.
3. Assessing the quality of teaching in a Classroom and Determining the merits of a particular work for Publication.

There are other examples where peer review is important such as research Accuracy, flawlessness of research studies, Journal Credibility, Positive feedback mechanism with Constructiveness.etc

Peer Review can be conducted in several different ways. They Include: -

1. Seeking the opinions of Individuals in a population, as in Human Epidemiological Research,
2. Using a panel of experts to select which research Proposal should be funded or writing an authoritative document that has wide implications,
3. Using Student opinions, coupled with those of the peer colleagues in determining the quality of teaching by an individual in a classroom or a predetermined view of the scientific part and
4. Using two or more experts in assessing the Quality of a manuscript for it's publication in a Journal.

Peer review is a mechanism for preserving the originality, authenticity, Validity and Quality of a particular work. It is also a way to prevent "Plagiarism". Certainly, some of those issues have led to controversy. Nevertheless, peer review is the standard and widely accepted method for evaluating the quality of the papers submitted for publication, even in the electronic publishing process. It is a Trustworthy tool for Academics; it improvises research integration and efficiency.

Peer Reviewing is of many types like Open Review (Both Author and the Reviewer know each other or identify each other), Single blind review (only reviewer knows the author and not vice- versa) and Double-Blind Review (Both Author and the Reviewer do not know each other or do not identify each other).

Similarly, there are various stages of Peer reviewing, starting from submission of the literature or text followed by Editor's Selection or screening, reviewer's evaluation or scrutiny followed by decision making in positive or in negative whether or not paper has to be published.

The Roles of various stake Holders in paper reviewing is very clear cut and distinct where the writer has to present a good manuscript, the reviewer has to deliver an honest, fair opinion and the Editorial board has to take the final Judgement regarding Publication. Peer Reviewing helps improvement in quality of research, helps in construction or build of an academic image, facilitates scientific conversation, and confirms Scientific attitude and integrity.

Peer reviews have their own problems. In some cases, there can be a conflict of interest or a pre- determined scientific view on the part of the reviewer that may influence his or her review. This is called as Reviewer's bias. Many peer review processes require the reviewer to disclose such information *a Priori,* so that such individuals are not included in the overall process. Failure to disclose such information can frequently lead to disservice and penalties. Another source of the problem can be, when the Editor of the Journal who serves as the Adjudicator errs in selecting the Reviewers.

Although many peer reviewers are dedicated and take their tasks very seriously, others do not. Many Universities do not offer points or Institutional rewards for the time spent in peer reviewing. That makes it difficult for young Faculty members seeking Promotion. How can the Authors of the paper get any more unsolicited help? It is through Peer reviewing that some Peer reviewers write comments that are aimed or directed at making the paper better.

In contrast, some potential reviewers never respond to a request *a Priori* for assistance, in spite of repeated queries. However, once agreed, the reviewer should provide his or her comments expediently. There are reviewers who neither provide their comments nor return the copy of the manuscript after agreeing to do the review. That is a total disservice to the authors and to the scientific Community. As such things happen, names of the individuals involved are removed from future consideration for requesting help. This is because peer review preserves the quality of science and you owe it to your colleagues to be helpful to them. Other issues regarding Reviewing include absence of error proofing of the script, time consumption, delayed publication on account of multiple suggestions and retakes, etc.

Peer Reviewing can be enhanced by using AI tools for a specific portion of a paper say mathematics, statistics, data, etc. for that part of the manuscript that has gone unnoticed by the human eye. In Future, AI holds a great potential for the same. But this does not mean that AI shall be replacing the peer reviewer. Peer Reviewing Supports

Collaborative research or forms a base for Multidisciplinary Research. . It is for this reason that peer reviewing is gaining popularity day by day.

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12. Traditional and Emerging Risk Factors of Coronary Artery Disease with a Focus on Obstructive Sleep Apnea

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1. Introduction

Coronary artery disease (CAD) is the leading cause of death worldwide and a major contributor to disability-adjusted life years (DALYs). The disease arises from progressive atherosclerosis of the coronary vessels, leading to impaired myocardial perfusion and clinical outcomes such as angina, myocardial infarction, and sudden cardiac death. Traditional risk factors for CAD, such as hypertension, diabetes, and dyslipidemia, have been well characterized through landmark epidemiological studies including the Framingham Heart Study. However, increasing evidence points to additional non-traditional and emerging determinants—including inflammatory biomarkers, genetic predispositions, and lifestyle-associated factors—that also modulate disease progression.

Among these emerging risk factors, **obstructive sleep apnea (OSA)** has attracted growing attention. OSA is a sleep-related breathing disorder marked by recurrent collapse of the upper airway during sleep, resulting in intermittent hypoxia, sleep fragmentation, and exaggerated sympathetic activation. Its role in cardiovascular disease, particularly CAD, has been strongly supported by epidemiological and mechanistic studies. This chapter reviews both traditional and emerging risk factors of CAD, highlighting the pathophysiological and clinical relevance of OSA as a novel contributor to coronary disease burden.

2. Traditional Risk Factors of CAD

Risk Factor	Mechanism	Impact on CAD	Key Reference
Age & Sex	Hormonal changes, endothelial senescence	Increased prevalence with age; higher male risk until menopause	Benjamin *et al.*, 2019

Hypertension	Shear stress, arterial remodeling	Linear rise in CAD mortality	Lewington *et al*., 2002
Dyslipidemia	LDL oxidation, foam cell formation	Strong causal factor in atherogenesis	Ference *et al*., 2017
Diabetes	Hyperglycemia, glycation, oxidative stress	2–4× risk increase	Emerging Risk Factors Collaboration, 2010
Smoking	Oxidative stress, platelet activation	Direct endothelial injury; reversible with cessation	Ambrose *et al*., 2004
Obesity & Sedentarism	Insulin resistance, systemic inflammation	Amplifies multiple pathways	Lavie *et al*., 2009

2.1 Age and Sex

The incidence of CAD rises steeply with advancing age. Men develop CAD earlier than women, though female risk accelerates post-menopause due to reduced estrogen-mediated vascular protection (Benjamin *et al*., 2019).

2.2 Hypertension

Hypertension exerts continuous mechanical stress on vascular endothelium, leading to dysfunction, arterial remodeling, and accelerated atherosclerosis. Longitudinal data confirm a log-linear association between systolic blood pressure and coronary events across all age groups (Lewington *et al*., 2002).

2.3 Dyslipidemia

Elevated low-density lipoprotein cholesterol (LDL-C) is causally linked to atherosclerosis through subendothelial lipid deposition and foam cell formation. High-density lipoprotein cholesterol (HDL-C) is inversely associated with CAD, though its role may reflect reverse cholesterol transport rather than absolute levels (Ference *et al*., 2017).

2.4 Diabetes Mellitus and Insulin Resistance

Diabetes accelerates atherosclerosis through chronic hyperglycemia, oxidative stress, and glycation end-products. Insulin resistance contributes to endothelial dysfunction and a prothrombotic state, explaining the two- to four-fold increased CAD risk in diabetics (Emerging Risk Factors Collaboration, 2010).

2.5 Smoking

Tobacco exposure induces oxidative stress, systemic inflammation, platelet aggregation, and endothelial injury. Smoking cessation rapidly reduces CAD risk, demonstrating a strong causal relationship (Ambrose *et al*., 2004).

2.6 Obesity and Physical Inactivity

Central obesity promotes dyslipidemia, hypertension, and insulin resistance, amplifying CAD risk. Sedentary lifestyle further aggravates cardiometabolic dysfunction (Lavie *et al*., 2009).

3. Emerging and Non-traditional Risk Factors

Emerging Factor	Mechanism	Clinical Impact	Reference
Inflammation (hs-CRP)	Cytokine-driven endothelial damage	Independent predictor of CAD	Ridker *et al*., 2016
Lipoprotein(a)	Pro-atherogenic, pro-thrombotic	Strong genetic determinant	Tsimikas *et al*., 2017
Hyperuricemia	Vascular dysfunction, hypertension	Marker of metabolic syndrome	Borghi *et al*., 2018
Homocysteine	Endothelial injury, thrombosis	Linked with folate/B12 deficiency	Wang *et al*., 2016
Psychosocial Stress	Autonomic imbalance, non-adherence	Raises event risk	Rozanski *et al*., 1999
Environmental (PM2.5, noise)	Oxidative stress, inflammation	Higher CAD mortality in polluted regions	Brook *et al*., 2010

3.1 Inflammation and hs-CRP

High-sensitivity C-reactive protein (hs-CRP) is a robust biomarker of systemic inflammation. Prospective studies demonstrate that elevated hs-CRP predicts CAD events independent of cholesterol levels (Ridker *et al*., 2016). Inflammation is now viewed as a causal mechanism in atherogenesis.

3.2 Lipoprotein(a) [Lp(a)]

Lp(a) is a genetically determined lipoprotein particle with pro-atherogenic and pro-thrombotic properties. Elevated Lp(a) levels increase CAD risk irrespective of LDL-C levels and are particularly relevant in individuals with premature disease (Tsimikas *et al*., 2017).

3.3 Hyperuricemia

Elevated serum uric acid has been associated with hypertension, endothelial dysfunction, and CAD. While the causal role remains debated, uric acid is increasingly considered a metabolic risk marker (Borghi *et al*., 2018).

3.4 Homocysteine and Micronutrient Deficiencies

Hyperhomocysteinemia damages endothelial cells and promotes thrombosis. Deficiencies of folate, vitamin B12, and vitamin D may contribute to this pathway (Wang *et al*., 2016).

3.5 Psychosocial Stress and Depression

Chronic stress and depressive symptoms influence autonomic tone, inflammatory activation, and adherence to therapy, thereby elevating CAD risk (Rozanski *et al*., 1999).

3.6 Environmental and Social Determinants

Air pollution, noise exposure, and socioeconomic disparities significantly modulate cardiovascular outcomes. Fine particulate matter (PM2.5) has been linked to higher CAD incidence (Brook *et al*., 2010).

4. Obstructive Sleep Apnea (OSA) and CAD

4.1 Epidemiology

OSA prevalence is estimated at 9–38% in the general population and exceeds 50% among CAD patients (Senaratna *et al*., 2017). Importantly, OSA is often underdiagnosed in cardiac patients, limiting opportunities for timely intervention.

4.2 Pathophysiological Mechanisms

- **Intermittent Hypoxia and Oxidative Stress**: Recurrent desaturation-reoxygenation cycles enhance production of reactive oxygen species, promoting lipid peroxidation and endothelial dysfunction (Lavie *et al*., 2003).
- **Sympathetic Overactivity**: OSA induces nocturnal and daytime sympathetic activation, raising blood pressure and heart rate variability (Somers *et al*., 2008).
- **Inflammatory Pathways**: Intermittent hypoxia upregulates pro-inflammatory cytokines (IL-6, TNF-α) and activates nuclear factor κB (NF-κB), driving vascular injury (Ryan *et al*., 2005).
- **Endothelial Dysfunction**: OSA impairs nitric oxide bioavailability and flow-mediated dilation, resulting in arterial stiffness and plaque formation (Jelic *et al*., 2008).
- **Prothrombotic State**: Increased platelet activity and fibrinogen levels in OSA patients foster thrombosis, exacerbating CAD risk.

4.3 Clinical Outcomes

OSA is independently associated with incident CAD, accelerated progression of coronary atherosclerosis, and adverse outcomes after myocardial infarction and percutaneous coronary intervention (Lee *et al*., 2016; Marin *et al*., 2005).

4.4 Treatment Implications

Continuous positive airway pressure (CPAP) therapy improves blood pressure control, endothelial function, and quality of life in OSA patients. However, large randomized trials such as the SAVE trial reported no significant reduction in major cardiovascular events, highlighting issues of adherence and patient selection (McEvoy *et al*., 2016). Alternative strategies, including mandibular advancement devices and weight loss, may complement CPAP in CAD patients with OSA.

5. Conclusion

Coronary artery disease represents the culmination of complex interactions between traditional cardiovascular risk determinants and newly recognized metabolic, inflammatory, and environmental influences. While traditional factors such as hypertension, diabetes, dyslipidemia, and smoking remain central to prevention strategies, the recognition of nontraditional contributors—such as Lp(a), systemic inflammation, psychosocial stress, and environmental exposures—broadens our understanding of atherosclerotic risk beyond classical models.

Obstructive sleep apnea (OSA) occupies a unique position within these emerging risk factors. It is highly prevalent, underdiagnosed, and mechanistically linked to CAD through a constellation of biological pathways, including intermittent hypoxia, oxidative stress, endothelial dysfunction, and systemic inflammation. Unlike many genetic or demographic risk determinants, OSA is potentially modifiable, making it an important therapeutic target.

The clinical evidence underscores that OSA not only coexists with CAD but also worsens its trajectory, influencing both incident disease and outcomes after interventions. However, the partial failure of CPAP to reduce cardiovascular endpoints in large randomized trials points toward the complexity of translating pathophysiological insights into therapeutic benefit. Issues of adherence, heterogeneity of OSA phenotypes, and interactions with other comorbidities must be considered in future clinical strategies.

In summary, CAD prevention in the 21st century requires a dual strategy: continued vigilance for traditional risk factors and active incorporation of emerging determinants into risk assessment frameworks. Screening for OSA in CAD patients, supported by public health measures such as community-level screening programs, awareness campaigns, and lifestyle interventions, can help bridge clinical insights with broader preventive strategies. Future research should focus on integrating molecular biomarkers, genetic predictors, and novel imaging tools to refine the relationship between OSA and CAD and to identify patients most likely to benefit from targeted interventions.

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13. Emerging Water Scarcity and Importance of Rainwater Harvesting as Sustainable Management Strategy

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Abstract

Fresh water availability is an escalating global issue, due to population growth and unsustainable practices that has a deep impact on human as well as plants and environment. Keeping this situation in mind, this chapter discusses emerging water scarcity, its causes and threatening effects. To overcome this problem and to save the future generations from water shortage, proper distribution and management of water resources are essential. This chapter also discusses the importance of rainwater harvesting as a sustainable management strategy. Various methods like rooftop system, surface runoff collection, construction of check dams etc. are used to collect the rainwater, which can be used as an alternative source of water in water deficient areas for irrigation, ground water recharging and for domestic activities. Though the use of these methods has increased a lot, its utility can be enhanced by improving and regulating water policies as well as by creating public awareness for water conservation.

Keywords: *Water scarcity, Rainfall distribution, Sustainable water management, Rainwater harvesting methods, Ground water recharge, Sustainable development goals.*

1. Introduction

Water is one of the five elements found in our body, which forms the basic foundation of life and is essential for the survival and maintenance of all living beings. Human being consume the water in various activities and it is directly or indirectly related to all type of human activities. In India per person water requirement is about 135 liters per capita per day (lpcd) in urban areas and 55 lpcd in rural areas as per Ministry of housing and Urban Affairs (PIB, Delhi 2020), so demand of water is going to be increasing day by day. It is necessary for all the issues such as climate change, food production, industrial development, energy production and environment conservation. However, due to the increasing population, industrialization and development the demand of this natural resource outweigh its supply. With only 4% of world's water resources, India has about 16 % of population, which leading to water shortage. Water stress, unavailability of water and water shortage are included in the concept of water scarcity. This could be the result of both anthropogenic and natural activity. The primary causes of this problem are inadequate resource management, insufficient focus, and sewage water produced by human. Over the last ten years, official data shows that the country's yearly per capita water availability has drastically decreased, leaving 163 million Indians without access to safe drinking water (Singh, R.,2019). In our country the distribution of water and rainfall is very irregular and unpredictable (Basistha, A., 2007). Some parts of the country receive heavy rainfall throughout the year such as Meghalaya, Mizoram, Assam while others receive low rainfall like Rajasthan, Haryana etc. Mawsynram of Meghalaya receive the highest rainfall whereas Jaisalmer district of Rajasthan receive lowest rainfall, which is responsible for the different climatic conditions of the states. Due to these differences some parts of the country facing the problem of flooding while other facing the scarcity conditions. Both of these conditions are not good, as in some parts drinking water is not available sufficiently and the people have to struggle for water while others from heavy rainfall areas have to leave their house due to flood. Therefore to overcome this problem, water should be used sustainably. Sustainable use refers to the process, which can be sustained for a longer time. Sustainable resources should be used in a way that avoid creating sustainability gap by overutilizing natural resources of earth. We should also keep in mind the rights of future generations so that natural resources can be made available to them too. Rainwater harvesting systems are also used as an imortant sustainable management strategy. Rain water harvesting methods collect different amounts of water depending on the weather and location. In some states like Himachal Pradesh, Uttarakhand, Haryana, these methods are widely used and legally supported while in other states though this technique is used but not as common as in above states. Rain water has both potable and non-potable use. Drinking, bathing, cooking, and cleaning are all considered as potable applications. Rain water utilized for this purpose typically needs to be treated to get rid of impurities. Toilet cleaning, garden watering and floor washing are examples of non-potable usage for which rain water treatment is not necessary. The rainwater increases the water level by ground water recharging, but due to improper drainage system most of rainwater become waste and cause soil erosion. Water and environmental conservation, pollution reduction, flood control, and lessening effects of climate change are the primary benefits of rain water harvesting systems.

2. Water Scarcity

Water has been functioning as an important factor since the dawn of civilization. Its importance is mentioned in our religious and cultural texts, where it is worshipped as a deity. Apart from this, its importance has also been described in various articles, films, songs and arts. Water is a basic need to life and livelihood and is necessary for sustainable development. Human existence, atmosphere and economic growth all depend on it. A livable wage combined with properly managed working conditions can give employees a steady source of money, lead towards more extensive growth and development but nowadays it is emerging as a major challenge for 21 century, as the demand for water increasing continuously. The phenomenon demand of fresh water is outstripping its supply due to increased population, urbanization, pollution load, water intensive agricultural activities, climate change and exploitation of ground water resources, affecting the communities and economies of the world. Every person needs water for their socioeconomic development. People have right to get water in appropriate quality and quantity as per their general drinking water requirement. The adoption of a basic water need benchmark of 50 liters per person per day by various state and central governments, global organizations and water suppliers ensure availability to drinking water regardless of social, political and economic standing of the person. If this fundamental need is not satisfied, widespread human suffering will persist, which will lead to worse conditions in the years to come. (Lal,B.S.,2019). The efforts of central and state governments to make available safe water to people through various type of rules and regulations, use of scientific methods to prevent misuse of water and deterioration of water quality is a big challenge. Concern over the supply of water and the degradation of its quality is growing as human activities in the environments reach unprecedented heights. The amount and quality of water that is accessible are significantly impacted by the massive changes made to the water cycle and environment etc.(Singh, S. 2021). According to a report of UNESCO, around 2100 million individuals are not getting access to potable water. One in four primary schools do not have drinking water facilities, due to which children use polluted water or are left without water. Every year more than 700 children under the age of around 5 years die due to polluted water and diseases caused by it. Nearly 80% of people living in rural areas across the world use poor and polluted water. In water scarce areas, 8 out of 10 women and girls have to travel long distance for water storage. Nearly 159 million people get drinking water sources like ponds and rivers. 4000 million people, which is nearly 2/3 of the world population, face drought for at least one month of the year. By 2030, nearly 700 million people will be affected due to water shortage. These various reasons become the cause of water scarcity. In this chapter, the causes of water scarcity, its effects and ways of reducing water problem and its planned use have been described.

2.1 Causes of Water Scarcity

The situation, when the rate of water use overcomes the rate of water supply by nature, is called a water problem/ water crisis/ water scarcity/ water shortage. The water scarcity, arising due to water misuse, water will be considered as new oil in future. Various man made activities are leading to the process of water scarcity, which are as follows

a. Increased Population - India has become the most populated nation of the world. This rapidly emerging population is putting additional burden on the available water resources and leads to water scarcity. India has only 4% of the world's water resources, while population is 16 % of the world, due to which there is a water shortage .With the expanding population of world by around 80 million individual per year, need for available water is predicted to be rise by 64 billion cubic meters annually by 2025, resulting in water scarcity in 52 nations, that houses 23 of the world population.

b. Inefficient Use in Agriculture - India is an agricultural country where different type of crops are grown . Different quantities of water are required for the cultivation and production of crops, so around 85% of the water is used in most of the agricultural areas. Increasing population leads to increased food demand, so consumption of water is increasing. The kind of grains and crops, we are growing has a direct impact on the consumption of people. Generally, the production of different crops are determined according to their consumption and yield. Besides this, use of old and traditional irrigation methods in agriculture leads to low water production and water scarcity situation. Cultivation of water intensive crops like sugarcane, cotton, rice are also a cause of water shortage in certain states of India as Maharashtra, Punjab, Rajasthan, Bihar, West Bengal and sometimes this water is supplied from underground water resources.

c. Pollution of Water Sources - Due to various natural and human activities, the physical and chemical properties of water sources are changing, causes water pollution and leads to water shortage. Water pollution can occur from one to more sources. During rainfall various sediments (releases organic and inorganic matter) get mixed in the water of rivers and streams. Apart from this chemical effluents released from industries, factories, chemical waste, sewage water, domestic and raw waste, agricultural pesticides and different heavy metals from mining industries mixed into the water through surface runoff and pollute it, leads to water pollution. Water pollution affects the quantity and quality of water for human and environmental use. CPCB found that most of the rivers in India like Ganga, Yamuna, Narmada, Ghaghara are polluted. Weak enforcement of water conservation laws, ground water regulations and environmental laws has hampered efforts to alleviate water shortage.

d. Climate Change - Due to various anthropogenic processes, environmental conditions are changing and lead to climate change. Water resources are affected by changed weather, increased temperature. Irregular monsoon, drought and changing rainfall pattern have affected the availability of water in various areas. Climate change does not directly cause the variation in water availability, rather it is affected by the changing conditions of the earth system, leads to fluctuation in natural limits, which causes risk management of water cycle. Increased temperature leads to heat accumulation in the environment, which has a direct effect on the availability of water resources. According to Singh,S.(2021) hot atmosphere contain more water vapor. With an increase in temperature by one degree, leads the atmosphere to carry 7% more water vapors In India, there is a stellar distribution of rainfall, where most of the rain is received as monsoon weather. In some states like Meghalaya, Manipur, Assam, there is excessive rainfall whereas in some states like Rajasthan, Punjab, Haryana etc., there is a continuous shortage of water due to less rainfall.

e. Industrialization and Urbanization - Increasing population and development has increased urbanization and industrialization, due to which the demand for water has increased in urban areas and industrial zones, leads to many Indian cities to face the problem of water shortage. The effluent coming out from industries not only affect the surface water sources like rivers, streams but also contaminate ground water through leaching or percolation, has a harmful effect on the health of local individuals. Aquifers are being depleted due to excessive use of ground water for irrigation, industries and domestic purposes. According to the report Central Ground Water Board, India is excessively exploiting its ground water resources especially in Punjab, Haryana and Tamil Nadu.

2.2 Effects of Water Scarcity

a. Health Issues - Lack or unavailability to potable water can lead towards various health hazards such as dehydration, diseases, infections and even death. Use of polluted water can also lead to diseases like cholera and typhoid.

b. Effect on Biodiversity and Ecosystem - Water scarcity causes natural water resources to dry up, which poses a threat to aquatic organisms, wildlife and natural environment. Due to lack water wild animals move towards villages, can result in danger and harm. Water scarcity leads to death of aquatic animals, which has a direct impact on the ecosystem through food chains. Apart from this it also affects biodiversity.

c. Economic Loss - Water shortage can cause a big hinderence to the economic growth and development of India. Due to water shortage industrial production affected, energy production can decrease, water supply and treatment can increase. It can also affect tourism, business and social welfare.

d. Effect on Agricultural Production - India is an agricultural country, which can be negatively affected by water shortage as it uses 85% of its water in agriculture. Water scarcity will affect the production capacity of crops, crop yield will decrease, which will not be sufficient for the increasing population of the country and will lead to poverty and hunger. Apart from this farmers income will also be affected and their poverty can increase.

All of this is taking place in a nation where people have long worshipped water. It is paradoxical because while we believe in Jal Devta and see rivers as goddess, we are also contributing to the depletion of water as a renewable resource.

2.3 Corrective Measures to Reduce Water Scarcity

We depend on nature for water. Life without water is unimaginable, hence some corrective measures are adopted to manage the water scarcity, which are given below

a. Reducing Over Consumption of Water - Due to excessive and inefficient use of water in various areas home, agriculture, and industries , the shortage of water is increasing. To overcome this problem different modern technologies should be used, which will help in mapping of water consumption. These techniques can help farmers in providing weather information as well as irrigation processes, which will not only save water but also increase the crop yield. There should be restrictions and regulations for industries and factories as they cause water pollution by releasing effluents. Water consumption can be reduced by motivating farmers to produce low water consuming crops. Using irrigation systems like drip, sprinkler, and buried clay plantation technique (in dry areas of Rajasthan) can not only reduce the water quantity of water in agriculture but also increase the production. According to the report of M.S. Swaminathan Committee, use of these techniques can reduce the amount of water used in agriculture by 50% and crop production can increase upto 40 to 60% . Besides this water consumption can be reduced by proper management, improvement in existing water policies, increasing clear rules and incentives for water conservation, increasing people's participation and integrating water related issues in development plans.

b. Improving Water Management Skills - To overcome the water shortage, the performance of water systems and structures can improve, which includes distribution networks, treatment plants and storage facilities. Water wastage can be reduced and quality and quality can be improved by repairing water leaks, reducing losses and upgrading facilities. Water conservation and rain water collecting, renovating traditional and other water bodies, reusing water and recharging structure, watershed development and intense afforestation are five components that will be considered in the water conservation process (Singh, S. 2021). In addition to this traditional local knowledge, we can benefit from contemporary technology and cross border experiences. Israel, a nation with little rainfall, has set very high standards for water management and recycling and there is much to learn from them in this area. It has the highest water recycling rate in the world at 85%. Highest water efficiency in agriculture, 70 to 80% of the world, is made possible via drip irrigation. It boosts the largest sea water reverse osmosis facility in the world, which generates 100 million cubic meters of desalinated water a year at a reasonable price. (Singh, S.,2021).

c. Conservation of Water Resources - Water cycle can be managed only by conservation of natural resources of water like rivers, ponds, waterfalls, wetlands etc. and by reorganizing the water sources. Forest and soil conservation also play an essential role in managing the ecosystem as well as in reducing water pollution, preventing erosion and controlling drought and flood. Alternative or additional sources of water should be developed through increasing water harvesting, desalination process, aqueduct, water reuse and ground water extraction so that availability and accessibility of water can be increased. Along with this, emphasis should be given on using water in a sustainable manner, so that it can be preserved for the future as well.

3. Rainfall Distribution in Indian States

Rainfall is considered as an important source of water, which is essential for both agriculture and living beings. In our country variations are observed in the rainfall distribution pattern according to time and situation, so the rainfall is considered to be the most unpredictable hydrometeorological parameter (Basistha, A.,2007). Quantitative estimation of rainfall distribution is necessary for drainage management, hydrological modeling, flood and drought control, forecasting of weather, climate change studies and to find out the water balance level. Rain and its significance are mentioned in ancient Indian texts. In some texts, details about measurement of rain, arrival of monsoon and the path of monsoon clouds are also found. Monsoon and rain have also been presented beautifully in the fields of dance, music, arts, painting, poems and writings etc. The unusual distribution of rainfall in India is seen due to changing atmospheric conditions, topography, their distance from coastal areas and moisture containing winds. The Indian seasons are divided into four parts winter(Jan. to Feb.), Pre monsoon (March to May), Monsoon(June to Sep.), post monsoon(Oct to Dec.) , out of which approximately 75 to 80% rainfall received in various regions of the nation by south west monsoon/ summer rainfall, therefore it is important to study the rainfall distribution on different time scale (Deshpande and Kulkarni *et al*.,2011). Indian Meteorological Department works for the mapping and measuring the rainfall, which describes the complex variations in different parts of the country by using rain gauges. Rain gauges are used to measure rainfall and are used in places where wind does not affect the normal rain drop fall, provides more accurate and reliable data of rainfall. Over 6100 rain gauge stations are part of the program, which is used to create real time rainfall data for various rainfall parameters. This rainfall data provided by IMD help researchers to find out the temporal and spatial distribution of rainfall in different parts of the country (Guhathakurta, 2007). Rainfall normal have been calculated using daily rainfall statistics from a network of 4311 stations across the country spanning 50 years (1971 to 2020). Rainfall information in operational near real time are available on a daily, weekly, monthly, seasonal, and annual basis. This rainfall data is used to forecast weather conditions (flood and drought) , climate change, hydropower plant need, or making water conservation strategies. Apart from this, also used to study the pattern, quantity, distribution and impact of rainfall in agriculture, irrigation sector and drainage management on different time scale. Table 3.1 lists the seasonal and observed rainfall in millimeters with their categories in different states of the country. It was found that during the year, Goa received most area weighted rainfall (4960.5 mm), while Ladakh received least amount (79.3mm). Categories are prepared by comparing percentage departure of rainfall from normal rainfall , which are as following — Large Excess (≥60), Excess (20 to 59%), Normal (— 19 to +19%), Deficient (—20 to —59%), Large Deficient (—60 to —99%), No Rain (—100%). According to this data, 8 states had considerable large excess/ excess rainfall, 21 stayed in normal category, and 7 were in the deficient category, for yearly rainfall.

Sr. Number	**State/ Union Territory**	**Annual Rainfall in (mm.)**	**Category (percent departure)**
1.	Andhra Pradesh	1059.40	Normal
2.	Andaman Nicobar Island	2794.20	Normal
3.	Arunachal Pradesh	2192.70	Deficient
4.	Assam	1858.50	Normal
5.	Bihar	916.70	Deficient
6.	Chandigarh	870.10	Normal
7.	Chhattisgarh	1364.10	Normal
8.	Dadra Nagar Haveli and Daman Diu	2088.90	Normal
9.	Delhi	732.70	Normal
10.	Goa	4906.60	Excess

11.	Gujarat	1114.20	Excess
12.	Haryana	476.10	Normal
13.	Himachal Pradesh	979.00	Deficient
14.	Jammu Kashmir	873.10	Deficient
15.	Jharkhand	1183.00	Normal
16.	Karnataka	1303.70	Normal
17.	Kerala	2795.40	Normal
18.	Ladakh	79.30	Excess
19.	Lakshadweep	2144.10	Excess
20.	Madhya Pradesh	1224.40	Normal
21.	Maharashtra	1385.30	Excess
22.	Manipur	1303.90	Deficient
23.	Meghalaya	3446.40	Normal
24.	Mizoram	1886.50	Deficient
25.	Nagaland	1362.30	Normal
26.	Odisha	1372.90	Normal
27.	Puducherry	1757.60	Excess
28.	Punjab	391.50	Deficient
29.	Rajasthan	710.00	Excess
30.	Sikkim	2799.20	Normal
31.	Tamil Nadu	1172.70	Excess
32.	Telangana	1113.50	Normal
33.	Tripura	2282.90	Normal
34.	Uttar Pradesh	794.10	Normal
35.	Uttarakhand	1486.20	Normal
36.	West Bengal	1873.40	Nromal

Source Rainfall Statistics of India 2024

Tab3.1: State wise Annual Rainfall (in mm.) and Departure Category (%) of India (2024).

4. Rain Water Harvesting: As Sustainable Management Strategy

Nature has provided man with the essential elements of water, air, food and environment for life, which are essential for the development and growth of any civilization and culture. Nature has been providing life to people since ancient times and will carrying on in upcoming time also, it's called sustainability. The term sustainability is derived from the latin word "sustainere" which means "to keep, bottom" . Sustain can mean to support or to endure. Basically, sustainability means using natural, renewable resources in such a way that people can depend on them for a long time. A prerequisite for sustainability is the preservation of the entire available resources equals to or higher than the current standard. Development means bringing about socioeconomic improvement of people on large scale. This require providing with opportunities as well as various choices so that everyone's standard of living can be improved and future generations can also get benefits and opportunities (Rana and Guleria ,2018). 3 components of sustainable development are: use, save and know it, which lead human towards overall self-reliance, freedom and development. It not only makes people aware of the aspect of the development but also points out various dangerous situations of the future, so that the misuse of resources can be stopped. Sustainable development goals were implemented by the United Nations to increase the sustainable use of natural resources and to improve the living standards of the people of developed and developing countries, which will reduce penury, conserve the environment, fostering well-being and promoting hormony. In next 15 years, the goals will spur action in areas that are vital to the planet and to humanity. These are committed to prevent the world from deteriorating, which include managing its assets efficiently, eco-friendly consumption of resources and responsible production. There are 17 SDG working in a unified manner, so that the work of one sector will impact multiple sectors, helps in balancing social, economic, and environmental sustainability (sds.un.ac). SDG 3,6,11,13,15 are specifically related to the health, water availability, sustainable communities, climate change and life on earth.

Sustainable management of water can also be done through rain water harvesting, which has emerged as an alternative source. Rainwater harvesting stores water, which is later used for various purposes. Water conservation methods have been used since ancient times to prevent water from being wasted and to save it. In our country, water conservation methods are being used since time of "Cholas", they were adept in this work. They built such systems, through which water could be stored. An example of this is the Bruhadeshwar temple, where water was collected in a pond. In old times water conservation policies and measures were simple and easy, wherein rainwater was directly collected and without any treatment it was used as a potable and non-potable source. But currently, due to increasing population and economic development in the country, demand for water is continuously increasing, to fulfill this demand and to overcome water scarcity, use of rainwater harvesting system have been suggested by the experts and scientists working in this area, because some sources of water are limited and seasonal. The amount of water collected by rainwater harvesting system depends upon the location and atmospheric conditions, which vary in different parts of the country. Rainwater harvesting not only conserve water but also help in reducing pollution level, prevent soil erosion and regulate climate conditions. In rainwater harvesting, rainwater is stored during the rainy season through mechanisms designed and created by engineers, for which water storage tanks are installed along with proper rooftop and terrace management. This technique is usually used in residential areas. In some parts of our country like Tamil Nadu, Chennai, Banglore, Shillong, Indore, Bhuvneshwar, Haryana, Punjab, Himachal Pradesh and Uttarakhand, rainwater harvesting system is used a lot, for which various policies have also been made by the government. These policies not only promote water conservation but also save many waters deficient areas from drought by providing them water but in our state, Uttar Pradesh, rain water harvesting system not used on wide scale, for which rule should be made.

By saving water, rainwater harvesting system provides mankind with an alternative resource for a better future. According to a study, if even one third of the rainfall water is harvested, there will be no need of other water resources for a year. Water level can be increased by ground water recharging through rainwater harvesting by recharge pit, tube well, open well, trench, shaft, dug well etc. Apart from this collected water through various methods (rooftop system, down piping, gutters, water drain and filter chambers etc.) can be filtered and stored, which can be later used as per requirement. Along with this, rainwater harvesting system help in controlling urban floods, reducing natural resource dependency, rejuvenating aquifers or rivers and prevent climate change. Thus, rainwater harvesting involves the collection and storage of raindrop, so that it can be prevented from flowing away unnecessarily and can be reused.

4.1 Rainwater Harvesting Methods

The storage of rainwater is mostly done from roofs or directly through surface runoff. The size of rainwater harvesting systems and methods of collection may vary from place to place and also depends upon environmental conditions. It can be of small to medium size depending upon the catchment area. Medium size includes college and universities, army camps, airports, restaurants, shopping complexes etc. while small size includes roof of houses, parks, grounds. Along with this, open fields, dry rivers and ponds also store water. Some of the methods of rainwater harvesting are given below.

a. Rooftop System - This is the widely used method of rainwater harvesting, used in houses, schools, offices etc. In this, water is collected from the rooftops, sent down through pipes and stored in a storage tank. After treatment and filtration, it can be used for drinking purpose. When rainwater comes in contact with the collection surface, various types of bacteria, moulds, algae, protozoa and other contaminants also reach it. Some physical contaminants like organic and inorganic chemicals, heavy metals are also found, so before using this water its quality parameters are studied (Mohammed et. al., 2007). Various methods are used to treat or filter this water, including chlorination (to clean public water systems, not widely used due to harmful effects), ultraviolet light disinfection (water purification by certain wavelength of light to eliminate microbes), and distillation methods (eliminate impurities by boiling it and collection of condensed water etc. (Waseem and Ghazi *et al*., 2023).

b. Surface Runoff Collection - The water flowing from road, street, park, ground, courtyard, balconies and drains not only get wasted but also caused urban flooding and erosion. This runoff is diverted through various methods and sent to tanks, ponds, recharge wells and percolating pits.

c. Creation of Water Reservoirs - These are used to improve and increase the ground water level, wherein surface runoff is collected and directed into gravel/ sand pits, which slowly percolates, replenishes water level. Rainfall water collected in rivers, ponds, lakes, wetlands, during monsoon season, used in various domestic activities.To reduce runoff in urban areas, ecofriendly roof gardens are built, which absorb rain water. In rain gardens, water is collected in small vegetated depressions.

d. Formation of Check Dams - Due to heavy rainfall in hilly and rural areas, water level of rivers increases considerably, which affects life in rural areas. To prevent its wastage and store it for future use, dams are constructed. Water is checked and collected through dams, later used for irrigation and power generation as per requirement. Dams have been constructed on rivers through various projects, helps in water distribution, developing drainage and disaster management strategies.

4.2 Advantages of Rainwater Harvesting

a. In Human Well Being Through Sustainable Use - Water obtained through rainwater harvesting after filtration can be used for washing clothes and utensils, bathing, cooking and drinking, which can meet increasing demand for water supply and make clean water available to all, thereby promoting sustainable development. SDG 3 can be achieved by providing clean water and food to all, for which emphasis should be given on using rainwater after treating and filtering it, so that people can be protected from diseases caused by polluted water and food, lead towards a better quality of life. SDG 6 also ensures the availability of clean water and sanitation. Apart from this, sustainable cities and communities can be built through water supply and drainage management strategy (SDG 8). Climate change can be regulated by using water in a sustainable manner (13). Pollution can be reduced by controlling effluent release from industries and water used in processing operations, can protect the lives found in water (SDG 14). Life on earth can be lived in a planned manner by managing water conditions (SDG 16).

b. Irrigational Use - Rainwater can be used as a means of irrigation in agricultural areas, which not only reduces water consumption rate but also boost crop production. The quantity of rainwater used for irrigation depends on the duration, intensity, frequency and holding capacity of soil. When holding capacity is good, soil surface absorbs water quickly. Apart from this, plants found in irrigating area, their distance and size also indicate the use of rainwater quantity.

c. Ecological Restoration- Due to availability of water, survival rate of aquatic organisms increase and flora fauna flourishes, leads toward balanced ecosystem. Rainwater is a good source of water for plants due to low salt concentration, provides a good environment for plant growth. When collected rainwater percolates into deeper layers, salt concentration reduces, which enhances the root growth and water uptake capacity of plant.

d. Reduces Pressure on Natural Water Resources - Rainwater harvesting is an effective and alternative source of water conservation. Rainwater collected during monsoon season in natural water resources like rivers, ponds, lakes, canals and wetlands, is not only used by people in rural areas for domestic purposes, drinking by animals, agriculture but also reduces drought risk, thereby reducing water demand pressure on natural water resources. A well thought out policy can encourage rainwater gathering as a means of attaining sustainability and natural resource management globally (Mohammed *et. al.*) .It can be used for concrete and dust suppression on construction sites and in emergency conditions. It promotes sustainable architecture through ecofriendly building design and integration.

e. Mitigating Urban Flood - By collecting rainfall in urban areas through rainwater harvesting, various present and future problems can be avoided. Due to increase in population, urbanization, industrialization and rural to urban migration, drainage management does not work properly, which lead towards water stagnation in low lying areas and increases pollution load. RWH not only mitigate the risk urban flooding but also can be used as a source of water supply.

f. Groundwater Recharging-Surface runoff is collected and directed to tanks/ ponds, helps in ground water recharging through percolation. It is an easily accessible and widely used source of water in arid and semi-arid areas (water deficit areas) . Ground water recharging not only increases the water table but also works on soil erosion and land sliding, which promotes sustainable agriculture.

5. Conclusion

Water is required to fulfill every aspect of life but due to increased population, industrialization, pollution and rapid urbanization, water scarcity is emerging as a challenge. Its effect can be seen on our health, biodiversity, agricultural productivity, climate and ecosystem. Besides this, there is a lot of variations in rainfall distribution in our country, due to which some regions face drought and others face flood situation. Rainwater harvesting can be adopted as a sustainable and multi benefit strategy to reduce this irregularity, variation, and water scarcity risk. Sustainable development goals (3, 6, 11, 13, 14, 15, 16) can be achieved by providing sufficient and clean water to people. This water can be used in both potable (after treatment) and non-potable form. RWH is also beneficial in water percolation, preventing soil erosion, electricity production, industrial cooling, and filtration operations etc. It not only reduces water supply demand but also help in rejuvenation of dried natural water resources. Various methods of RWH are being used but to make it effective, along with improvement and regulation in water conservation rules, public awareness is also necessary, so that people understand the importance of water conservation and emphasize on its use with prudence.

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14. Bovine Tuberculosis in a Female Murrah Buffalo *(Bubalus bubalis)*: A Case Report

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Abstract

Bovine tuberculosis remains an economically and zoonotically significant disease in livestock. In addition to posing a risk to human health, it also leads to reduced productivity with higher rearing costs in livestock and the treatment is often not economically feasible. An eleven-year-old female buffalo was presented for postmortem with the history of marked submandibular and pharyngeal swelling accompanied by respiratory distress and inspiratory stridor. A complete necropsy was performed and revealed marked enlarged and firm retropharyngeal lymph nodes pressing the upper respiratory tract. Multiple firm, 5-10 mm caseous granulomatous lesions were also observed in the lung parenchyma. Histopathologically, the lesions were characterised by scattered to coalescing granulomas with calcification at the center. Modified Ziehl–Neelsen staining confirmed the presence of acid-fast *Mycobacterium bacilli*. The case was diagnosed as bovine tuberculosis based on gross, histopathology and acid-fast staining. This case highlights the role of conducting systematic necropsy and histopathology for the detection of mycobacterial diseases in large animals, especially in endemic regions.

Keywords: *Bovine tuberculosis, Buffalo, Granulomas, Mycobacterium bovis, Ziehl–Neelsen staining, Zoonosis*

Introduction

Bovine tuberculosis (bTB) is a chronic granulomatous disease of livestock caused predominantly by *Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex. The disease poses a dual threat to animal health and public health, given its zoonotic potential (Thoen *et al*., 2006). Economically, it is associated with significant losses due to reduced milk yield, infertility, poor growth, carcass condemnation, and premature culling (OIE, 2021). Buffaloes (*Bubalus bubalis*) are important dairy animals in many developing countries, yet tuberculosis in this species is often underdiagnosed due to its insidious course and limited routine diagnostic capacity (Francis, 1972). Transmission occurs primarily through inhalation of aerosols and, to humans, via contaminated milk and meat (WHO, 2020). This report documents a confirmed case of tuberculosis in a buffalo, with emphasis on clinical signs, pathological features, and laboratory confirmation.

Materials and Methods

An eleven-year-old female buffalo was presented with a history of progressive weight loss, chronic pharyngeal and ear base swelling, submandibular edema, respiratory distress, stridor and reduced milk production. Previous symptomatic treatments yielded no improvement. The buffalo was later found dead and subjected to a necropsy.

Post-mortem Examination: A complete post-mortem examination of the buffalo was carried out following standard necropsy procedures. All major organs were inspected carefully, with special focus on the lungs and lymph nodes because of the respiratory signs observed before death. Tissue samples showing visible lesions, particularly from the lungs, retropharyngeal lymph nodes and intestines, were collected for further laboratory investigation.

Histopathology: The collected tissues were immediately fixed in 10% neutral buffered formalin for adequate preservation. After fixation, the samples were routinely processed, embedded in paraffin wax, and sectioned at approximately 4–5 μm thickness using a rotary microtome. The sections were then stained with hematoxylin and eosin (H&E) to study the microscopic changes. Particular attention was given to identifying granulomatous inflammation, necrotic foci, and the type of cellular infiltration.

Ziehl–Neelsen (Z-N) Staining: For the demonstration of acid-fast organisms, impression smears were prepared from lung and lymph node lesions. Smears were air-dried, heat-fixed, and stained by the Ziehl–Neelsen technique. Carbol fuchsin was applied with gentle heating, followed by decolorization using acid-alcohol, and counterstaining with methylene blue. The stained smears were examined under oil immersion, where acid-fast bacilli appeared as slender, bright red rods against a contrasting blue background.

Results

Gross examination revealed firm, greyish-white nodules in the lungs and lymph nodes, containing caseous necrosis and calcification. Retropharyngeal lymph nodes were also enlarged, irregular with tuberculous lesions. (Fig. 1 and 2). Lung showed multifocal raised nodules (Fig. 3).

Histopathology of lung tissue and lymph node demonstrated typical granulomatous inflammation consisting of epithelioid macrophages, Langhan's-type giant cells, lymphocytes, and a necrotic centre with prominent calcification surrounded by fibrous connective tissue (Fig. 4-6). Ziehl–Neelsen staining of impression smear and formalin-fixed lung tissue revealed the presence slender, red-stained acid-fast bacilli (AFB) (Fig. 7). Thus, the case was diagnosed as bovine tuberculosis based on gross, histopathology and acid-fast staining.

Discussion

This case presents the classical clinical and pathological features of *M. bovis* infection in buffaloes. The findings are consistent with previous descriptions of bovine tuberculosis characterized by granulomas, caseous necrosis, and calcification in the lungs and lymph nodes (Corner, 1994). The zoonotic potential of *M. bovis* underscores the significance of this diagnosis. Infected buffaloes may act as reservoirs, transmitting infection to cattle and humans through close contact and unpasteurized milk consumption (Thoen *et al*., 2006). This case underscores the importance of routine herd screening, given the prevalence of subclinical infections

Diagnostic challenges remain a key obstacle. While tuberculin testing is widely used for surveillance, confirmatory diagnosis requires laboratory methods such as Ziehl–Neelsen staining, culture, and PCR (OIE, 2021). The use of molecular tools ensures rapid and reliable detection. This case highlights the continuing burden of bovine tuberculosis in buffaloes and reinforces the need for integrated control programs, including surveillance, farmer awareness, and biosecurity measures.

Conclusion

The present case demonstrates a confirmed occurrence of tuberculosis in a buffalo, with characteristic gross and histopathological lesions and laboratory confirmation of *M. bovis.* Given its zoonotic potential, bovine tuberculosis in buffaloes should always be considered in chronic respiratory illness cases. Strengthening surveillance, rapid diagnostics, and farmer education are essential for effective disease control

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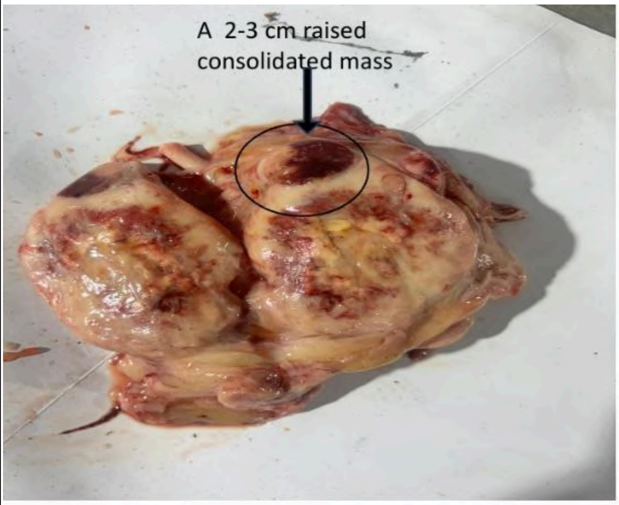
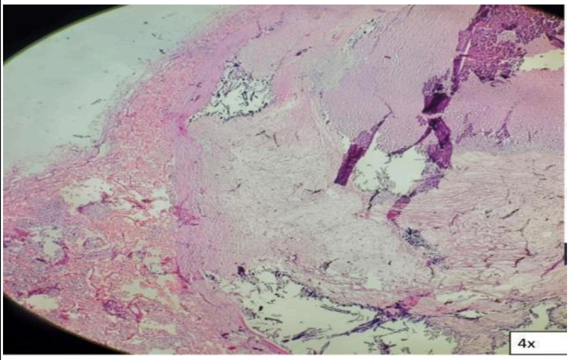
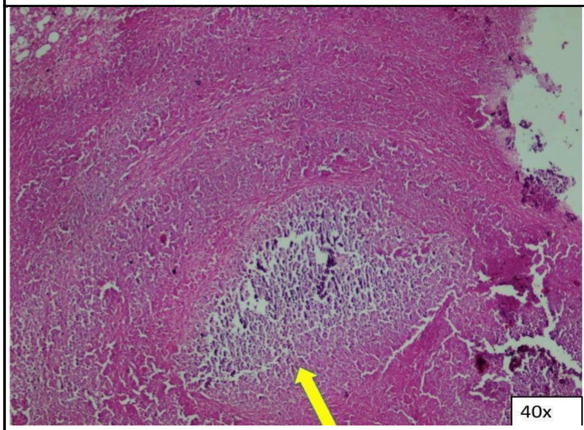
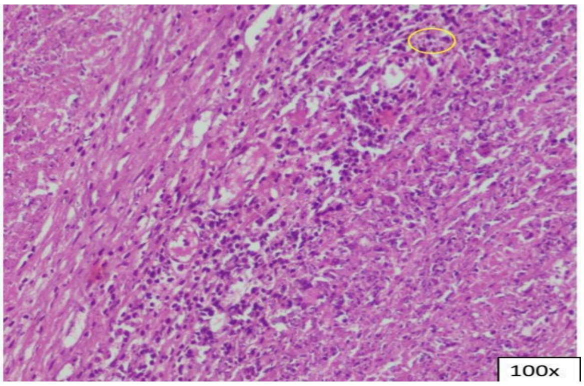
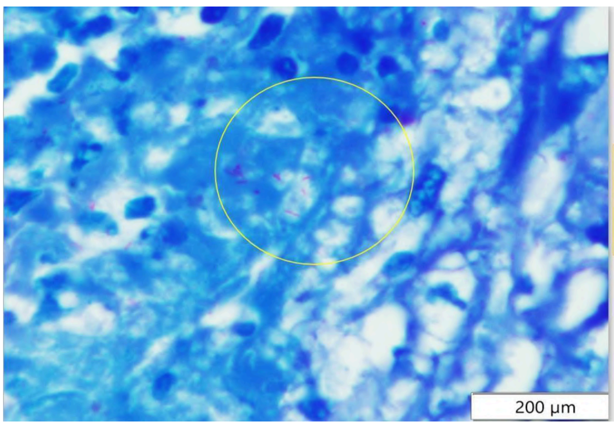
	Fig. 1: Image shows pair of hard ball shaped mass, irregular, firm to hard in consistency producing visible bulge behind pharyngeal wall found indicating enlarged retropharyngeal lymph nodes..
	Fig 2: Retropharyngeal lymphnode: Enlarged, irregular, firm, whitish-creamish with raised 2-3cm consolidated mass on the surface.
	Fig 3. Lung: Multifocal raised nodules (encircled).The reddish discoloration corresponds to areas of pulmonary haemorrhage.

	Fig. 4. Histopathology of lymph node showing the presence of granuloma .
	Fig 5. Lung: showing Centre area of necrosis (arrow) indicating calcification and presence of fibrous tissue surrounding granuloma .
	Fig 6. Higher magnification of image E showing infilteration of mononuclear lymphocytes and Langhan’s-type gaint cells (encircled) .
	Fig 7. Red color rods shaped *Mycobacterium in* observed in Z-N staining

15. Plant Taxonomy and Artificial Intelligence: A Comprehensive Review

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Abstract

Plant taxonomy, the science of describing, naming and classifying plants strengthens biodiversity research and conservation. However, traditional methods are labour-intensive and require expert knowledge, leading to a "taxonomic barrier" in understanding the world's flora. In the past decade, artificial intelligence (AI) and machine learning (ML) have emerged to aid taxonomy. Deep learning models, especially convolutional neural networks (CNNs), now achieve near-human accuracy in identifying plant species from images. AI also aids higher-level classification (genus, family), integrates genetic data (DNA barcoding) and unlocks massive digital herbarium datasets. This review surveys advances at the AI–taxonomy interface (2013–2024). We describe recent taxonomic innovations (molecular and integrative approaches), then detail how AI tools (image recognition, deep learning, data mining) are applied to all plant groups. We summarize key studies and tools, evaluate challenges (data gaps, model biases, interpretability), and outline future directions (associated learning, explainable AI, multimodal data). By combining AI with taxonomic expertise, researchers are accelerating species discovery and identification, ultimately making plant classification more scalable and comprehensive.

Keywords: *Plant taxonomy, artificial intelligence, machine learning, convolutional neural networks*

Introduction

Plant taxonomy involves identifying and classifying all plant species into a hierarchical system (species, genus and family) based on shared characters (Seeland *et al.*, 2019). It is fundamental to ecology, conservation, agriculture and medicine. However, taxonomy has long depend on expert knowledge of morphology and anatomy, making it slow and subjective (Hajam *et al.*, 2024). Globally, only about 390,000 plant species are formally described, and the backlog of un-described taxa creates a "taxonomic impediment"

(Nanni *et al*., 2024). This gap is exacerbated by declining numbers of taxonomists. Meanwhile, digitization has created vast image and genomic data (millions of herbarium scans on Global Core Biodata Resources, GBIF) (Walker *et al*., 2022), providing new raw material. Advances in evolutionary biology and high-throughput sequencing have revolutionized taxonomy; modern approaches integrate DNA sequences, biogeography and morphology to redefine relationships (Seeland *et al*., 2019). For example, molecular phylogenetics has reshaped family-level plant trees based on genetic similarity, sometimes overturning morphology-based classifications (Maltsev and Erst 2023). Despite progress, efficient identification of specimens (especially novel ones) remains challenging.

Artificial intelligence promises to help overcome these barriers. With affordable digital cameras, smartphones and big data, AI-based image analysis can automate species identification (Labrighli *et al*., 2022). Deep learning models can learn understated visual patterns from images of leaves, flowers or whole plants. AI also excels at analysing large genetic datasets (DNA barcodes) to predict taxonomic identity. Recently, mobile apps and online platforms (PlantNet, iNaturalist and Flora Incognita) have empowered citizens to contribute millions of plant photos, which in turn train machine-learning models. Early expectations that AI could match taxonomic experts are becoming reality. Current CNNs and ensemble models approach expert accuracy in controlled tests (Labrighli *et al*., 2022). This review examines the synergy of plant taxonomy and AI over the last ten years. We first outline our literature survey methods (Section "Methodology"), then review recent non-AI taxonomy advances, followed by how AI tools are used in taxonomy (image classification, genomics, etc.). We summarize findings from key studies and tools, discuss limitations (data gaps, model biases) and sketch future directions (multimodal AI). This work aims to inform plant biologists and AI researchers alike about the state-of-the-art at their intersection.

Methodology

We conducted a structured literature review of AI applications in plant taxonomy (2013–2024). Search terms included combinations of "plant taxonomy", "species identification", "machine learning", "deep learning", "image recognition", "DNA barcoding", and "phylogenomics". Databases searched were Google Scholar, PubMed, Scopus, and Web of Science. We included peer-reviewed articles and conference proceedings in English. Inclusion criteria required explicit use of AI/ML methods for identifying, classifying or demarcating plant taxa. We excluded studies on plant disease diagnosis, agriculture (unless taxonomic) or unrelated AI topics. Relevant review articles were also collected to understand context. From each source, we extracted data on (1) plant group studies, (2) AI techniques used, (3) data types and sample sizes and (4) key findings (accuracy, novel insights). Information was synthesized by theme (image-based ID, genetic classification, integrative methods, etc.). Table 1 summarizes our review workflow.

Step	Description
Literature search	Queried Google Scholar, PubMed, Scopus (2013–2024) using keywords "plant taxonomy", "machine learning", etc.
Selection criteria	Included AI/ML applied to plant ID/classification; excluded unrelated plant/disease papers.
Sources	Databases, journal publications, conference papers, and relevant preprints.
Data extraction	Recorded authors, year, target taxa, AI methods (CNN, SVM, etc.), data (images, DNA), and outcomes.
Synthesis	Organized content into sections (advancements, applications, tools and limitations) and compiled summary tables.

Table 1 Methodology of literature review and manuscript preparation

Recent Advancements in Plant Taxonomy

Beyond AI, plant taxonomy itself has progressed through new data and concepts. Integrative taxonomy now combines multiple evidence lines (morphology, DNA, ecology) to delimit species and higher taxa (Seeland *et al*., 2019). Molecular systematics such as analysing gene sequences has revolutionized phylogenetics, revealing new relationships unseen in classical morphology (Seeland *et al*., 2019). For example, DNA-based trees often rearrange family/order boundaries and cryptic species (morphologically similar but genetically distinct) have been uncovered. Rapid techniques like genome skimming and plastid phylogenomics generate huge genetic datasets for building robust plant trees. Meanwhile, massive digitization of herbaria and field surveys has expanded taxonomic databases: GBIF alone now aggregates ~400 million plant specimen records (Walker *et al.*, 2022). Big data projects (iPlant, 1KP transcriptome project) enable large-scale comparative analyses. Automated tools (high-throughput phenotyping) extract traits (leaf shape, flower structure) from images for large taxon sets. Cladistic and integrative frameworks have matured, proposing formal species hypotheses from multiple data sources. These advances mean taxonomy is no longer solely a manual discipline but increasingly data-driven. However, integrating diverse data remains complex. In this context, AI techniques add new capabilities by rapidly learning from heterogeneous data. Figure 1 showed various visual data (flowers, fruit, leaf and bark) now used for AI-based identification.

Despite advances, challenges persist in taxonomy. Many regions and lesser-known groups (ferns, bryophytes and algae) remain under-studied.

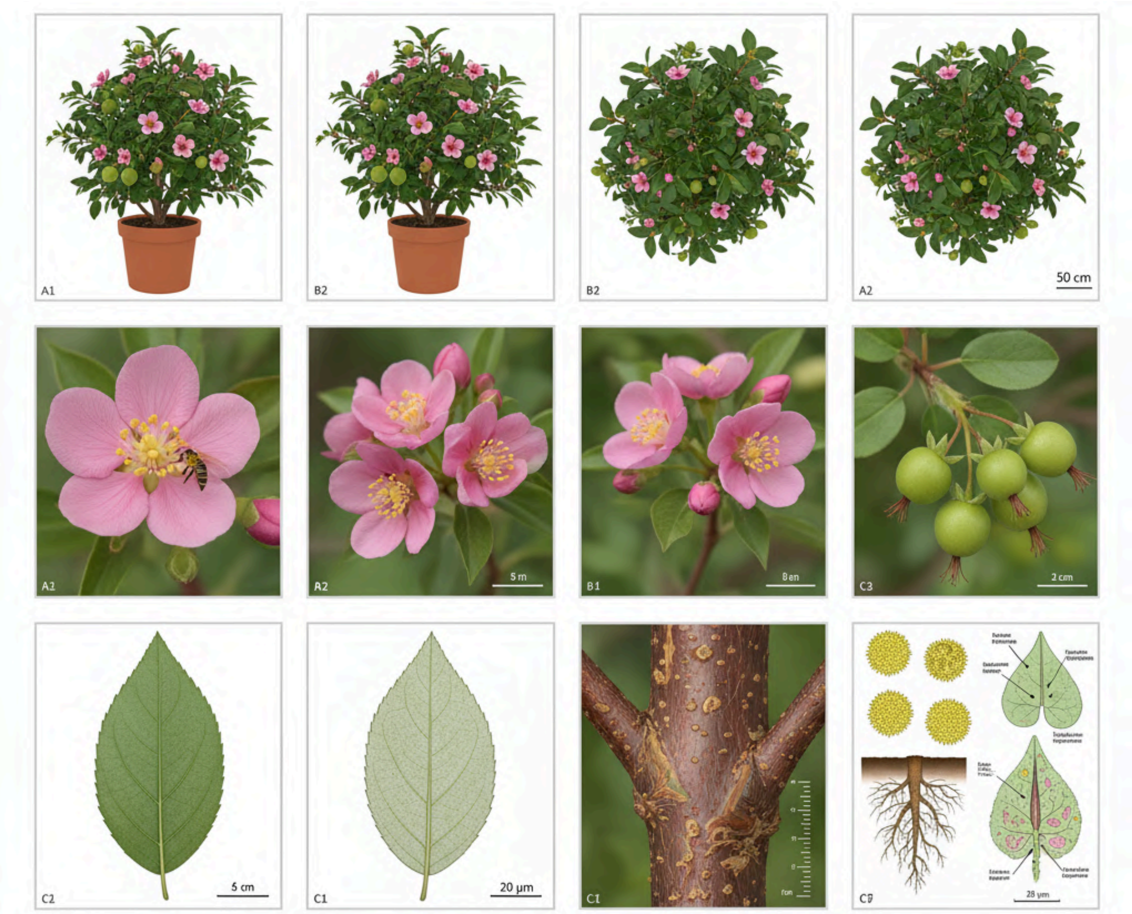

Figure 1 A single plant observed from multiple angles and organs (flower, fruit, leaf, bark) as in the PlantCLEF database. Such multi-view images are used in AI systems to capture diverse taxonomic features.

Data gaps in remote or mega diverse habitats hinder complete catalogues. Morphological convergence (unrelated species looking alike) can mislead classification. Species delimitation, especially with on-going speciation or hybridization, is still debated. Moreover, the shortage of taxonomists and funding continues to limit pace. Thus, augmenting human efforts with AI is viewed as a way to accelerate taxonomy without sacrificing precision.

AI Tools in Plant Taxonomy

Artificial intelligence has become a powerful aid in taxonomy by automating pattern recognition and data integration. Key AI techniques include traditional machine learning (SVM, Random Forest, kNN) and modern deep learning (CNNs, vision transformers, deep ensembles). Table 2 lists prominent AI-based tools and platforms used in taxonomy. In practice, AI assists taxonomy in several ways:

Image-based species identification: Convolutional neural networks (CNN) trained on leaf; flower or whole-plant images can rapidly classify species. For instance, CNN models achieve >90% accuracy on standard leaf databases (Turkoglu *et al*., 2021). State-of-the-art CNNs (ResNet, EfficientNet) and Vision Transformers have been applied to large datasets (PlantCLEF, iNaturalist) with excellent results (Picek *et al*., 2022).

Tool/Platform	**Description**	**AI/Tech Details**	**Country of Origin/Launch Year**
Pl@ntNet	Smartphone app + website for plant ID	Uses CNN models trained on multi-million image database; suggests species from photos (over 1.5M contributors).	France (2009)
iNaturalist	Citizen science platform for species observations	Leverages user-uploaded photos and location data; includes a “computer vision” AI that suggests species names.	United States (2008)
Flora Incognita	Research-based plant ID app	Deep CNN trained on Central European flora;	Germany (2018)

	(Germany/Europe)	achieved ~85% species-level accuracy in tests.	
Leafsnap	Mobile app identifying trees by leaves	Early CNN system trained on herbarium and field images of tree leaves; introduced leaf venation analysis.	United States (2011)
GBIF	Global Biodiversity Information Facility	Aggregates 400M+ plant records (including ~2M herbarium images); provides open APIs for data mining.	International (2001)
TensorFlow / PyTorch	Machine learning frameworks	Popular libraries for building and training CNNs, transformers, and other AI models for plant image or sequence data.	United States (2015/2016)
SHAP / LIME	Explainable AI toolkits	Used to interpret AI predictions (feature importance on images or gene data).	United States (2017/2016)
Leaf Venation Mapper (LeafVeinCNN)	Image analysis tool for leaf architecture	Uses ML to segment and quantify vein patterns, aiding taxonomic character extraction.	United Kingdom (2020)

Table 2 Advanced AI-based tools and platforms for plant taxonomy

Taxonomic level classification: AI also works at higher ranks. For example, classifiers can learn visual patterns characteristic of genera or families. Seeland *et al.* showed genus-level accuracy ≈86% and family ≈88% on 1000 European species (Seeland *et al.*, 2019). Such models exploit the fact that shared features (leaf venation, flower structure) exist within taxa. Notably, AI accuracy often increases at higher taxonomic levels because inter-family differences are more pronounced (Seeland *et al.*, 2019).

Genomic and integrative methods: Machine learning is applied to genetic data for taxonomy. Recent studies convert DNA barcode sequences into input for deep networks, achieving state-of-art performance. Nanni *et al.* (2024) transformed DNA bases into numerical images and used an ensemble of deep neural nets to classify species from barcodes (Nanni *et al*., 2024). These methods bypass manual tree-building and can handle cryptic species. AI is also used in phylogenomics to integrate multi-gene data and identify new clades (an emerging research area).

Herbarium digitization and trait extraction: Large image collections of pressed specimens have been leveraged by representation learning. Walker *et al.* trained deep "triplet" networks on millions of herbarium images to learn features useful for classification and information retrieval (Walker *et al*. 2022). AI can automatically measure traits (leaf area, vein patterns) from images, feeding into taxonomic analyses. Automated OCR and text mining of specimen labels is another AI-enabled task (not taxonomy per se, but improves data) Figure 2.

Mobile apps and citizen science: AI powers field identification apps. Platforms like Pl@ntNet, iNaturalist, and Flora Incognita use deep learning to suggest species from user-taken photos (Picek *et al*., 2022). These apps incorporate contextual data (location, time) to filter likely species. For example, the Flora Incognita app (CNN-based) correctly identified 80–85% of Estonian wild plant images to species (Pärtel *et al*., 2021). Such tools both educate the public and generate labelled data.

Explainable and interactive AI: Recognizing the need for interpretability, researchers apply Explainable AI (XAI) methods such as SHapley Additive exPlanations (SHAP) and Local Interpretable Model-agnostic Explanations (LIME) to explain plant ID predictions (Mishra *et al*., 2025). This allows taxonomists to validate AI decisions by highlighting image regions or gene motifs that drove classification. Interactive platforms use active learning, where the model queries experts on uncertain cases, improving its taxonomic knowledge base.

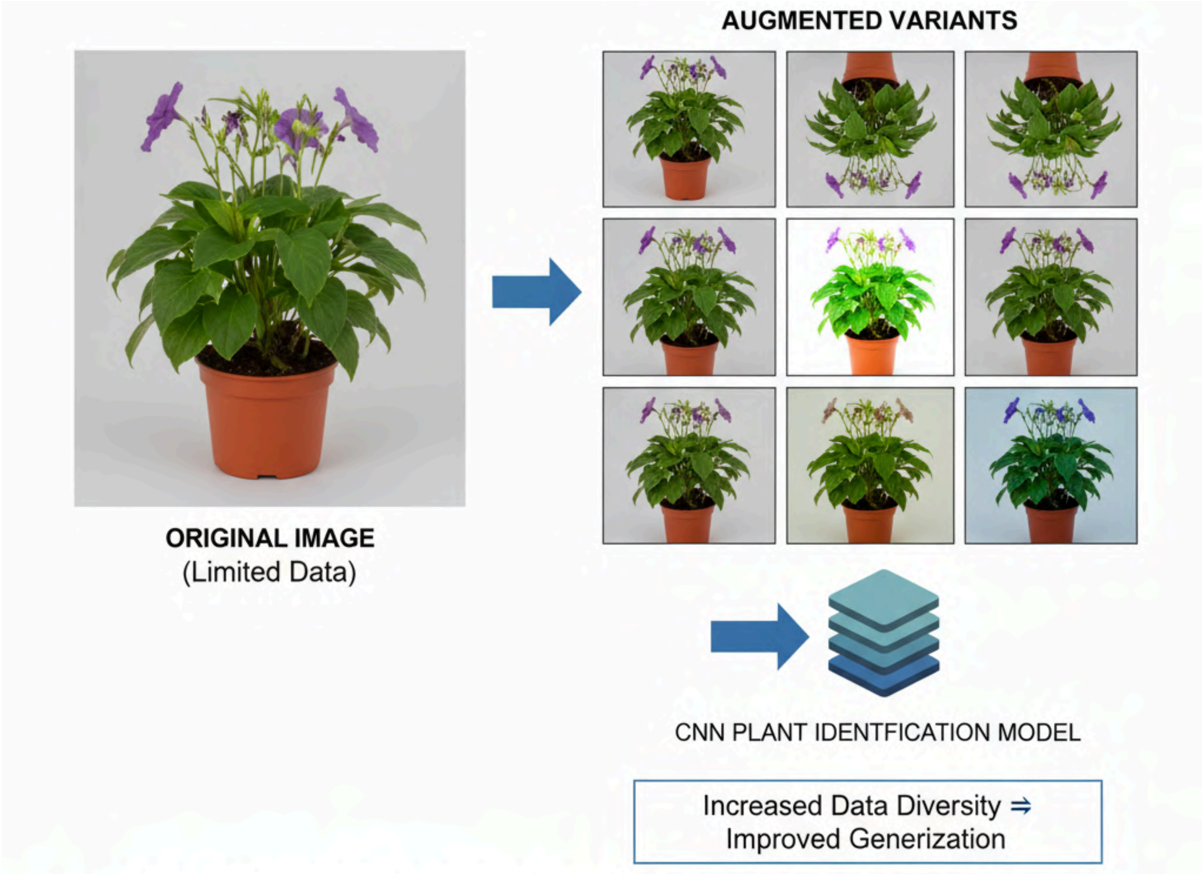

Figure 2 Data augmentation example used in training plant identification models. The original image (left) is transformed into multiple variants (right) via flips, crops, and color/brightness adjustments. These augmentations help CNNs generalize from limited training data.

Advanced tools: Table 2 lists prominent AI-driven tools and resources for plant taxonomy, including mobile apps, databases, and AI frameworks. For example, GBIF and naturalist provide massive crowd sourced datasets of plant observations (Picek *et al.*, 2022). The PlantCLEF challenges offer curated image datasets that push state-of-art algorithms (2015–2023 saw CNN accuracies rise from ~45% to 65% on hard benchmarks (Pärtel *et al.*, 2021). Powerful open-source libraries (TensorFlow, PyTorch, scikit-learn) and high-performance computing (GPUs) underlie these applications. As an example of innovation, the AI-powered **Leafsnap** and **Leafsnap Mobile** projects use CNNs trained on herbarium images to identify tree species by leaf (Mishra *et al.*, 2025).

In sum, AI tools enable high-throughput, data-driven taxonomy. They automate identification tasks, reveal hidden patterns in big datasets, and engage non-experts in science. As algorithms improve and data grow, AI increasingly complements traditional taxonomy.

Literature Survey of AI in Plant Taxonomy

A growing body of research demonstrates AI's role in taxonomy. Representative studies illustrate the diversity of approaches and results (Table 3). For example, Turkoglu *et al.* (2021) developed a "multi-division" CNN: they subdivided leaf images into patches, extracted features with a CNN, then classified with SVM. This hybrid MD-CNN+PCA+SVM system achieved nearly 100% accuracy on standard leaf datasets (Flavia, Swedish, Folio) and >94% on complex flower datasets (Turkoglu *et al.*, 2021). Seeland *et al.* (2019) focused on higher taxonomy; using ~500 images/species of 1000 Western European plants, a CNN learned visual genus- and family-level patterns. The model attained 82% species-level accuracy, rising to 85.9% at genus and 88.4% at family (Seeland *et al.*, 2019). Notably, for species unseen during training, accuracy dropped (38–39%) highlighting generalization limits (Seeland *et al.*, 2019).

Study (Year)	Target	AI Methodology	Key Findings
Turkoglu *et al.* (2021)	Leaf and flower images (floral datasets)	Multi-division CNN (patch-based) + SVM	~100% accuracy on Flavia, Swedish, Folio datasets; >94% on Flower17/102
Seeland *et al.* (2019)	1000 European plant species (natural images)	CNN-based classification	82.2% species, 85.9% genus, 88.4% family accuracy; unseen species <40%
Pärtel *et al.* (2021)	Northern European flora (Estonian data)	Flora Incognita app (deep CNN)	79.6% species accuracy (database), 85.3% (field); genus 89%, family 95%
Walker *et al.* (2022)	Global herbarium specimens (~2M images)	Deep triplet network (representation learning)	Learned general features; improved retrieval/classification across collections
Nanni *et al.* (2024)	DNA barcodes (various taxa)	Ensemble of deep neural networks	Achieved state-of-art accuracy in species classification using DNA barcodes

Table 3 Representative studies of AI in plant taxonomy (2013–2024). AI methods include CNN (convolutional neural net), DNN (deep neural net) and SVM (support vector machine)

Wäldchen *et al.* (2021) tested a practical app-based system. Using the Flora Incognita CNN on ~280 species from Estonia, they achieved ~79.6% correct species IDs from a curated database and ~85.3% in the field (Pärtel *et al*., 2021). The genus and family were correctly predicted for 89% and 95% of specimens, respectively. They found that identification success correlated strongly with the number of training images per species (Pärtel *et al*., 2021); common plants were easier to recognize.

Nanni *et al.* (2024) approached taxonomy via genetics. They transformed DNA barcodes into "images" by encoding nucleotide pair physicochemical properties and then trained an ensemble of CNNs. On benchmark and simulated datasets, this method achieved state-of-art performance (Nanni *et al*., 2024). This demonstrates that deep learning on genetic data can automatically classify species, offering a complement to conventional phylogenetic methods.

Walker *et al.* (2022) targeted herbarium images. Using a triplet neural network, they learned embedding's from millions of specimen photos that generalized across tasks. Their representation-learning approach improved classification and retrieval of herbarium data (Walker *et al*., 2022).

These studies, among others, show that AI yields high accuracy and scalability. They cover all plant groups (woody and herbaceous species, local floras to global trees) and a range of AI tools (CNN, SVM, ensemble DNNs). The cumulative literature suggests AI is rapidly becoming integral in modern taxonomy.

Shortcomings and Future Perspectives

Despite successes, AI in plant taxonomy faces limitations. A major issue is data dependency. Deep models require large, balanced datasets, but many plant species are rare or lack digital images. For example, Seeland *et al.* found that species not represented in training dropped to ~38% accuracy (Seeland *et al*., 2019). Similarly, Pärtel *et al.* observed that species with few training photos had lower ID success (Pärtel *et al*., 2021). Imbalanced data also bias models toward common taxa.

Another challenge is fine-grained classification. Plants often have subtle morphological differences, and AI can confuse closely related species. Current CNNs perform extremely well on curated images, but struggle more "in the wild". While scanned herbarium images yield ~99% ID accuracy, cluttered field photos are still hard (Picek *et al*., 2022).

Variations in lighting, background, or plant orientation can mislead algorithms. This suggests a need for robust augmentation and better generalization strategies.

Generalization to unseen species is also hard. Most AI models are trained on a fixed set of species, when encountering a novel plant; the system has no built-in mechanism for "unknown class" detection, risking false identifications. Hybrid methods such as clustering embedding's or using open-set classifiers are still in early stages.

Computational resources are another constraint. Training large CNNs or transformers on millions of images or sequences demands powerful GPUs and time. This can limit access for many researchers. Moreover, the "black box" nature of AI raises trust issues: taxonomists may hesitate to accept AI suggestions without interpretability. Tools like SHAP/LIME help by highlighting image regions or genetic motifs used for decisions (Mishra *et al*., 2025), but broader adoption is needed.

Future directions include integrating multi-modal data: combining images, DNA, geography, and climate in unified AI models may improve accuracy and reveal deeper taxonomic patterns. Federated learning (training across distributed datasets without sharing raw data) could leverage global herbarium collections while respecting data ownership (Mishra *et al*., 2025). AI can also assist "virtual herbaria" by linking specimens across institutions and detecting duplicates or mislabelled entries. Explainable AI must advance so users understand model outputs.

Ethical and social considerations are emerging as AI training data may have geographic biases (over-sampled regions/species) and conservation applications must respect indigenous knowledge and data sovereignty (Mishra *et al*., 2025). Finally, new AI paradigms like self-supervised learning or foundation models could learn taxonomic concepts from unlabelled data, potentially identifying cryptic species groups without explicit labels.

In summary, while AI greatly enhances plant taxonomy, it is not a magic bullet. Effective use requires high-quality data, expert validation and awareness of biases. On-going research is addressing these issues and we expect AI–taxonomy to become more robust and representative in coming years.

Conclusion

The last decade has seen remarkable convergence of plant taxonomy and artificial intelligence. AI methods, particularly deep learning on images and sequences, can now classify plant taxa with accuracy approaching that of human experts. New tools and datasets from mobile ID apps to sequenced genomes fuel this progress. This review has shown that across all plant groups, AI assists at multiple taxonomic levels, as summarized in Tables 2–3. However, key challenges remain in data coverage, model generalization, and interpretability. The future lies in integrative AI that combines morphological and molecular evidence (phylogenomic networks), improved data sharing and explainable systems tailored for taxonomy. By complementing traditional expertise with AI, researchers can accelerate species discovery, update classifications and make taxonomic knowledge accessible to all. Continued collaboration between taxonomists and computer scientists will be crucial to fulfil AI's promise in documenting Earth's botanical diversity.

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16. Review on Some Natural Remedies for Hormonal Imbalance in Female

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Abstract

Today's, if we look at our busy lifestyle schedule, it is necessary that we first focus on our hormone health. The most efficacious things you can do for maintain hormone health are in the preference and solutions you make day to day life. One of most important is adequate hydration of body, focus on take always healthy foods, maintain your healthy weight with exercises & movements, reduce stress, support quality sleep & gut health. Other than it, present study also focusing on some herbal medicine having no side effects such as sulforaphane, chasteberry, saw palmetto, maca, black cohosh etc. Many herbs and its constituents are supporting hormonal balance and its metabolism. Herbs, nutrient and its alternative treating & resolving many types of symptoms associated with hormonal imbalance. A naturopatheic doctor exclusively working in hormone health is well accomplished in herbal treatments that addresses common issues.

Keywords: Hormonal health, natural remedies, adequate hydration, reduce stress & herbal medicines.

Introduction

The endocrine system circulates hormones with blood to its target tissue or glands and performing various different functions throughout the day. Even minor alterations in level of hormones can have negative impact, involving extra stress, effect ×on the body. A overall health significantly affected by hormonal imbalance. Some factors are beyond a individual control. However, making some dietary daily lifestyle changes can help to restore a healthy hormonal balance of the body (Jon Johnson, 2025). Some hormonal imbalance can lead to abdominal weight gain or hormonal belly (Zawn Villines, 2024). According to the report of office women's health (OWH), approx 1 in 10 females are affecting with PCOS in her reproductive age. Sex hormonal levels are also changes overtime, but some of the most significant changes happen during puberty, pregnancy & menopause (Jamie Eske & Mandy French, 2024).

According to the report of Ignacio *et al* (2023), HBC (hormone- based contraception) disrupts the women's hormonal health and creating threatening artificial states of inoculation, that causes physiological, psychological and behavioral consequences. Norah (2022) was stated that because of social and reproductive role of women's, female bodies are rendered as susceptible to imbalance. Even the ageing process is entirely comlicated, and can pretentious a variety of hormone that are important for metabolic health, physical performance, body composition and perception (Mark *et al*, 2021). According to the research of Zeng *et al* (2022) were preferably chosen those drugs in clinical trials that have more efficacious and having the collective synergistic effects than an individual herbal agent for the management of PCOS e.g., leaves of *Astragalus caprinus* and Diane-35 or contraceptive pills and myo-inositol when used collectively in combination and proved to be more efficacious.

(A) <u>Daily routine activities</u>

The most important things you can do for hormonal health balance are in the choices you make daily in life.

1. Adequate Hydration - Water plays a crucial or essential role for good health.

2. Healthy Foods - Always focus on to take foods healthy. A low carbohydratic, highly protein rich, whole meal diet, helps to maintain blood glucose level & keeps you always fully balance.

3. Exercise & Movements - Even slow walking helps to balance your hormones & maintain your body weight. Exercises also release stress & help to regulate your mood throughout the menstrual & menopause phase.

Yoga reduce cortisol, B.P and blood sugar level. Malasana or garland pose (asana) are very good for hormonal imbalance condition. Even mediation, reading, listening music, journaling & coloring can also help to keep stress in check.

Most effective therapy for women with PCOS, especially for correcting imbalance in hormone levels related to it are doing regular yoga practices (Renuka & Jhansi, 2023).

4. Gut Health - Gut bacteria also changes hormonal balance & affecting the severity of PCOS, menopausal and endometriosis. Fiber rich foods feeds good bacteria in our intestines and supporting the elimination of excess hormones & toxins.

5. Reduce Stress - Normal function of estrogen, progesterone, testosterone & thyroid hormone is influences by stress. Under extremist stress & anxiety our adrenal glands releasing cortisol, that inhibiting the production of insulin hormone. Unbalanced of cortisol hormone occured due to impact of stress on adrenal gland functions. Low intensity exercise can help reduce the elevated level of cortisol hormone. Uniquely treatment may require by individuals with hormonal abnormalities. In some circumstances, surgical surgery may be required for the tumors like issues of endocrine system (Elissa, 2022). Neuroendocrine tumors, grow from neuroendocrine cells, which are present throughout the body. As neuroendocrine cells are present in most organs, these NE tumors can develop in many different areas of the body & cause different symptoms (Caitlin Geng, 2024).

6. Support Quality Sleep - Always trying to establishing a regular bedtime. At least one hour before sleep, shut downing your electronics items. Understanding your circadian rhythms & rehearsing of good sleep hygiene may help with hormone balance.

(B) <u>Some Herbal remedies</u>

Herbs, nutrients & supplementation can treat & resolving symptoms related with hormonal imbalance. Many weeds, herbs, plant compounds & its constituents support hormonal balance and its metabolism such as few are given below:-

1. Sulforaphane from seed extract of broccoli, aids hormone clearance through the liver (Micknovicz *et al*, 1997 & Dalessandri *et al*, 2004). This herb is knowing as diindolylmethane or DIM.

2. Chasteberry (fruit of *Vitex agnus* castus) **-** It used traditionally to support production of progesterone while reducing estradiol. Its berries also contained diterpenoid compounds, potential effects on prolactin & dopamine neurotransmitter (Cecillia Snyder, 2023). It has a very prominent effect on the pituitary gland, and has the potential to treat PCOS symptoms like amenorrhea, anovulation, and pelvic pain. But its use is prohibited during pregnancy or those taking birth control pills and also in patients taking parkinson's medications or antipsychotics (Westphal *et al*., 2006; Goswami *et al*., 2012).

3. Saw palmetto (*Serenoa repens*) - it helps to eliminate excessive testosterone & prohibit the by products that induce acne and hair growth, commonly observe with PCOS.

4. Maca (*Lepidium peruvianum***)** - It benefits during menopausal transition (Messiner *et al*, 2006). It can also help to maintain hormonal balance during the menstrual cycle, perimenopause & menopause phase.

5. Black Cohosh (*Cimicifuga racemosa*) influences hot flashes and other symptoms of perimenopause & menopause. It also helps to used manage breast cancer like symptoms (Shams *et al*, 2010). Black cohosh root having estrogen like effects, treating side effects of menopause and supporting women's reproductive health (Cecillia Synder, 2023).

6. Ashwagandha (winter cherry; *Withania somnifera*) overcomes stress and normalize the blood cortisol & thyroxine level.

7. Nigella Seeds (*Nigella sativa*) - Also known as fennel flower or kalonji. It contain a thymoquinone compounds which act as a very good antioxidants and helps to manage PCOS.

8. Marjoram (*Origanum genus*) - It contains bioactive flavonids & phenolic acids compounds that influence cortisol, estradiol & insulin level.

9*. Glycyrrhiza glabra* is used in various clinical conditions, such as an osteoarthritis, demulcent & expectorant, to treat various infections. It is used as an adjuvant in various therapies, but it also found to be very effective to reduce the serum concentration of testosterone and against hirsutism in PCOS patients (Yang *et al.*, 2018).

10. *Aloe vera* - Maharjan *et al* (2010) was checked the activity of *Aloe vera* against PCOS in a rat model and reported that it restores the steroid in ovaries and altered the steroidogenic activity & estrus cyclicity.

11. ***Linum usitatissimum*** **(**Flaxseed) - Its powder extract also shows very promising results in PCOS patients by reducing hirsutism & androgen levels (Nowak *et al.*, 2007).

12. Nabiuni *et al* (2015) was observed the effect of ***Curcuma longa*** leaves on the polycystic ovary in a female rat, decreasing the androgens level and showed improvements in ovulation.

13. ***Actaea racemosa*** is used to treat various medical conditions related to disturbance of hormonal level e.g., anxiety, mood swings, abdominal cramps related to periods, and menopause (PMS) etc. However, some negative side effects are associated with its use such as headache, muscular pain, obesity, GIT issues, and vaginal spotting (Fan *et al.,* 2021).

14. ***Paeonia lactiflora* -** When it is used daily in the form of tea, it regulates prolactin and estrogen secretion. It contains various phytochemicals constituents that help in hormonal regulation (Takahashi and Kitao, 1994; Westphal *et al*, 2006).

15. *Urtica dioica* - In patients with syndrome of polycystic ovary, having a very low level of the sex hormone-binding globulin (SHBG) while the production of the male hormone testosterone is more. This plant especially its root extract is used to lowering the testosterone level and elevated the production of SHBG to correct the hormonal imbalance in PCOS patients, but long-term use of this plant is harmful & can cause hypotension like condition (Najafipour *et al*., 2014; Zare *et al*., 2015).

16*. Camellia sinensis* (Green Tea) - Green tea is a very commonly used herbal remedy for weight loss (Ghafurniyan *et al.*, 2015). It modulated the gonadotropin level, reduced IR, and also improved the morphology of ovarian. Moreover, it reduced the ovarian cyst (Ghafurniyan *et al.*, 2015).

17. ***Silybum marianum* -** Manneras *et al*, 2010 was found that it to be very beneficial in regulation of hormone but its combination with metformin it proved to be more effective in treating PCOS symptoms, such as anovulation.

18. *Gymnema sylvestre* (Retz.) It is a traditionally knowing herb due to its antidiabetic and lipid-lowering action. Its antidiabetic activity is more probable due to its nutritive restoration action on the *beta*-cells of the pancreas. It also regulates insulin level and lowering the increased triglycerides level related to PCOS. Its active constituent is gymnemic acids i.e. saponin. If we take gymnemic acid before a meal, it masks the sweet sensation by suppressing the taste. It also regulates blood glucose in hyperglycemic patients (Hywood, 2004; Khanage *et al.*, 2019).

19. *Trifolium pratense* (**Red Clover) -** It phytochemical isoflavones are responsible to increases the progesterone level in the body. It also used to detoxify body & for the treatment of acnelike symptom of PCOS.

20. ***Sesamum indicum* (Sesame Seeds) -** Its seeds contain many beneficial nutrients like lignans, phytosterol, vitamins B1, B6, calcium, magnesium, and zinc etc that help in hormonal balance and are very effective in the management of PCOS. Its black seeds increase the insulin absorption while reducing the testosterone levels, and to regulate menstruation (Goswami *et al*, 2012; Ghasemzadeh *et al*., 2013).

21. ***Cucurbita pepo (Pumkin seeds)* -** It seeds contains omega 3 fatty acids that are very useful for hyperinsulinemia and to regulate high cholesterol levels. It is found to be very effective in eradicating the symptoms associated with polycystic ovary. It is also found to be a very rich in source of beta-sitosterol constituent i.e. involved in the reduction of excess testosterone (Szczuko *et al*., 2017).

22.*Oenothera biennis* (Evening Primrose Oil) - Studies found that the phytoestrogenic property present in its oil, are very effective as they act on the hypothalamic-pituitary axis and has wonderful effects on lowering the level of luteinizing hormone/FSH and testosterone (Meletis and Zabriskie, 2006; Zand Vakili *et al*., 2018).

23*. Serenoa repens (W.Bartram)* - Hirsutism is among one of the major symptoms of PCOS that occurrence due to the increased production of estrogen; in addition, it reduces obesity and increases libido because of its antiandrogenic activity (Vassiliadi *et al.*, 2009; Nagarathna *et al*., 2014).

24.*Tribulus terrestris* L. (Puncture Vine) - *Tribulus terrestris* L. is very effective in increasing ovulation, treating menstrual irregularities, and also has an antidiabetic property (Arentz *et al*., 2014; Parikha and Krishna, 2019).

25. *Mentha spicata* L. (Spearmint Tea) - This tea having some antiandrogenic properties (Grant, 2010; Goswami *et al.*, 2012).

26. *Matricaria chamomilla* L. (Chamomile) - Scientist results demonstrated that *Matricaria chamomilla* leaves can decreased the total testosterone levels while raised the LH level (Amir*et al.*, 2007).

27. *Astragalus dasyanthus* - *Astragalus* polysaccharide helps in the metabolic regulation of PCOS symptoms. It is more beneficially when used in combination with Diane-35. (Luo *et al*., 2009; Nagarathna *et al*., 2014).

28. *Foeniculum vulgare* (fennel) - It has been traditionally used drugs, for the treatment of infertility & anovulation. It has highly antioxidant, anti-inflammatory, estrogenic properties due to which it has the potentiality to treat PCOS (Karampoor *et al*., 2014).

29. *Ferula asafoetida* - Ghavi *et al*., 2020 were also studied the effects of *Ferula* on ovarian structure and androgenic hormone level in PCOS patients.

(C) <u>Some other natural remedies</u>

1. *Cinnamomum verum* (Cinnamon)- Are found to be very effective in improving IR and potentiating insulin action and It also very helpful to cure hormonal imbalance by increasing the progesterone level and decreasing the testosterone level production in females (Sun *et al*., 2004).

2. Ginger - It also improves insulin resistance in letrozole induced PCOS.

3. Oats - Are the good breakfast for energy & hormone balance.

4. Berries, apples & pomegranates are fruits that help with hormonal balance. Berries rich in antioxidants that can help to fight against inflammation (Sahoo *et al*, 2024). Pomegranates can help with estrogen balance by supporting liver- health, help to lower the risk of inflammation & act as a good source of natural plant based estrogen.

5. Dried Fruits - Almonds, walnuts, pistachios & dried apricots are rich in protein, fibre, antioxidants & healthy fats. They also regulate blood sugar, managing PCOS and reduce inflammation. Also help to regulate hormone balance.

6. Brown rice - Rich in fibers, vitamins & minerals.

7. Coconut Milk - Can help balance hormones.

8. Lemon water - K+ in lemons help with brain & nerve function, which can increase your alertness. Some scientists found that just a whiff of lemon can boost your Immunity & you feel good hormones are working and reduces stress levels.

9. Even **Turmeric drink** also balanced & maintaining your healthy diet.

10. Eggs - It benefits the human body especially hormones. Eggs are rich in protein and helps to maintain level of insulin and ghreline secreation in the body.

11. Fish oil & Flax - Its supplements rich in omega- 3 fatty acids, may caused inflammation & alleviate discomfort associated with endometriosis, menopause, PCOS, & premenstrual syndrome, inflammation often triggers pain etc.

12. Spearmint tea - Benefits for women dealing with hormonal imbalance such as PCOS.

13. Vitamin D - Good source from sunlight, regulate estrogen production and its activity depending on situation.

14. Magnesium - Helpful to supports body biochemical reactions from liver detoxification to cell energy production. Also used as natural muscle relaxant (for cramps), promote digestive health, treat headaches; where blood sugar is often a concern having potential to improve insulin sensitivity in patients with PCOS (Kostov, 2019).

Avoid item's

Avoiding following drinks to take, that plays a very active role for hormonal imbalance:-

1. Green Tea - Intake of green tea disturbs the level of estrogen in case of hormonal imbalance.

2. Coffee & Caffeine - Can affect hormones in a number of ways. They decreases the level of estradiol and enhance the secretion of cortisol or increases level of stress hormone, that distrupts body's hormonal balance.

3. Diet Soda or Fruit juice increases the estrogen secretion in females.

4. Milk products - Avoide dairy products to take. They are rich source of calcium, but its high intake can lead to gut inflammation & irritation.

5. Quitting smoking & alcohols - Very actively involve in hormonal imbalance of the body.

6. Avoids oils high in omega - 6, especially when cooking.

7. Avoid over eating.

8. Avoid too much light at night, take high quality sleep.

9. Avoid to eat red meat, soy products and limit processed, canned & junk foods etc. They having high amount of saturated fats & elements that can influences hormonal balance.

Conclusion

In females, menstrual disorders are the most prominent sign of hormonal imbalances such as PCOS, amenorrhea, stress, premenstrual syndrome and other related issues etc. Due to high economic costs and a high number of unfavorable effects associated with the use of allopathic medicines, the demand of herbal medicines are increased. Herbal plants remain a major source of medicinal preparation from ancient times (Arentz *et al*., 2014). Regardless of the great revolution in the pharmaceutical field, the trend of using herbal medicines is increasing day by day exceptionally in developing countries, (Iqbal *et al*., 2022). This review article is useful in understanding the properties and importance of herbal medicinal plants, that are playing a very crucial role to cure symptoms of PCOS. We are also discuss about all the easily available, cost-effective herbal drugs with a potential effect. A holistic routine, proper diet, healthy lifestyle, some natural & herbal remedies can help you to recover from the hormonal health issues. The strategies, may beneficial for some people, but who is concerned about their hormone issues should've consult a healthcare professional.

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17. Nature's Pharmacy And Perfume: A Comparative Study Of Medicinal And Aromatic Plants In India And Beyond

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Abstract

Medicinal and aromatic plants were studied for their therapeutic, cultural, and economic value in both Indian and global contexts. The aim was to compare traditional uses, modern applications, and cultivation trends. A survey was conducted among 200 respondents, including farmers, herbalists, and consumers, to assess awareness, usage patterns, and preferences. Indian species such as *Ocimum tenuiflorum* (tulsi), *Withania somnifera* (ashwagandha), and *Cymbopogon citratus* (lemongrass) were compared with global species like *Lavandula angustifolia* (lavender), *Panax ginseng* (ginseng), and *Men*-tha *piperita* (peppermint). Data analysis revealed that 78% of respondents used medicinal plants regularly, while aromatic plants were preferred by 64% for wellness and commercial purposes. Literature review findings confirmed that medicinal plants were historically embedded in Ayurveda, Traditional Chinese Medicine, and Western herbalism. It was concluded that combined promotion of traditional knowledge with modern scientific validation could enhance sustainable use and international trade. The results also suggested that government support, quality control, and farmer training were essential for large-scale cultivation. Both Indian and global case studies demonstrated that medicinal and aromatic plants had significant potential in health, cosmetics, and the export market.

Keywords: Medicinal plants, Aromatic plants, India, Global trade, Traditional medicine

Introduction

Medicinal and aromatic plants were recognized as vital resources for human health and economic development. Their use was recorded in ancient texts, folk traditions, and modern pharmacopeias. In India, the Ayurvedic system preserved extensive plant-based remedies, while globally, systems like Traditional Chinese Medicine (TCM) and

European herbalism maintained similar heritage. Over the last few decades, demand for plant-based products was increased due to the shift toward natural and organic remedies. Aromatic plants, valued for essential oils and fragrances, were used in perfumery, cosmetics, and aromatherapy. The global herbal medicine market was valued at billions of dollars, and India contributed significantly to raw material supply. However, challenges such as overharvesting, adulteration, and lack of quality control were faced in both domestic and export markets.

Objectives

1. To document the traditional and modern uses of medicinal and aromatic plants in India and globally
2. To analyze the market potential and trade of these plants.
3. To assess awareness and usage patterns among consumers and producers.
4. To compare cultivation practices and sustainability challenges.
5. To recommend strategies for sustainable promotion and global competitiveness.

Methodology

A mixed-method approach was adopted. Primary data were collected through a survey of 200 respondents from India and international participants in herbal trade expos. Secondary data were gathered from journals, WHO reports, and government statistics. The questionnaire included demographic details, plant usage frequency, source of purchase, and preference for Indian vs. global species. Data were analyzed using percentage analysis and comparative charts.

Literature Review

Studies on medicinal plants in India highlighted species like tulsi, neem, and ashwagandha for their antimicrobial, adaptogenic, and immune-boosting properties. Aromatic plants like lemongrass and sandalwood were studied for their essential oil content and fragrance industry applications. Globally, lavender and peppermint were reported for stress relief and digestive benefits, while ginseng was studied for energy enhancement and immunity. WHO reports indicated that 80% of the global population relied on traditional plant-based medicine for primary healthcare. Research also revealed that global demand for essential oils was growing at a rate of over 8% annually.

Despite extensive research, gaps existed in integrating traditional knowledge with modern pharmacological validation.

Case Study 1– Lemongrass Cultivation in Anantapur District, Andhra Pradesh

In 2021, farmers in Anantapur district of Andhra Pradesh shifted from rainfed groundnut cultivation to *Cymbopogon citratus* (lemongrass) farming under a state-supported aromatic plant promotion program. Training was provided by the Central Institute of Medicinal and Aromatic Plants (CIMAP). Lemongrass was cultivated on 60 acres of previously underutilized dryland. The crop required minimal irrigation and was harvested thrice a year. Essential oil extraction units were established locally, reducing transportation costs. Annual income per acre was increased from 25,000 (groundnut) to 55,000 (lemongrass). Farmers reported better drought resilience and steady market demand from herbal product manufacturers in Hyderabad and Bengaluru. The initiative was recognized as a model for sustainable aromatic plant farming in semi-arid regions of Andhra Pradesh.

Case Study 2 – Cultivation of Tulsi in Uttar Pradesh, India

In 2022, a farmer cooperative in Uttar Pradesh was supported by the National Medicinal Plants Board to cultivate *Ocimum tenuiflorum* (tulsi) on 50 acres of land. Organic farming methods were adopted, and high-yield seed varieties were distributed. The harvested leaves were processed into herbal tea and essential oils. Annual revenue was increased by 35% compared to traditional wheat farming. The project was highlighted as a sustainable model that provided both environmental and economic benefits.

Case Study 3 – Lavender Farming in Provence, France

In southern France, the cultivation of *Lavandula angustifolia* (lavender) was promoted as both a cultural heritage and an economic driver. In 2021, farmer associations collaborated with perfumery companies to improve quality standards and adopt eco-friendly distillation methods. The region ex- ported over 1,200 tonnes of essential oil annually, contributing significantly to France's aromatic plant industry. This success was attributed to consistent branding, strict quality control, and global demand for natural fragrances.

Survey

A total of 200 participants were surveyed: 120 from India and 80 from other countries. Respondents included 40% farmers, 35% herbal product consumers, and 25% traders.

Data Analysis (Tables & Graphs)

Table 1: Respondent Demographics

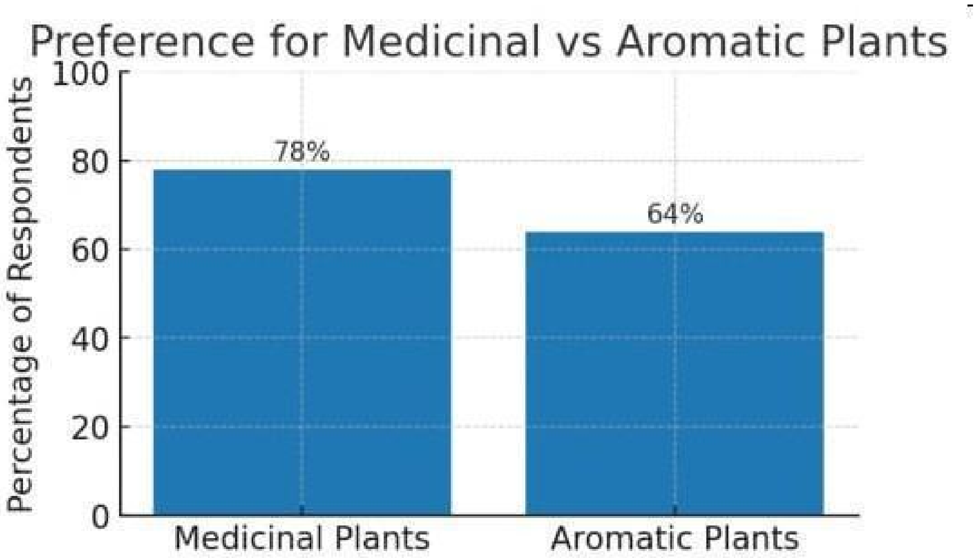
Category	Number	Percentage
Farmers	80	40%
Consumers	70	35%
Traders	50	25%

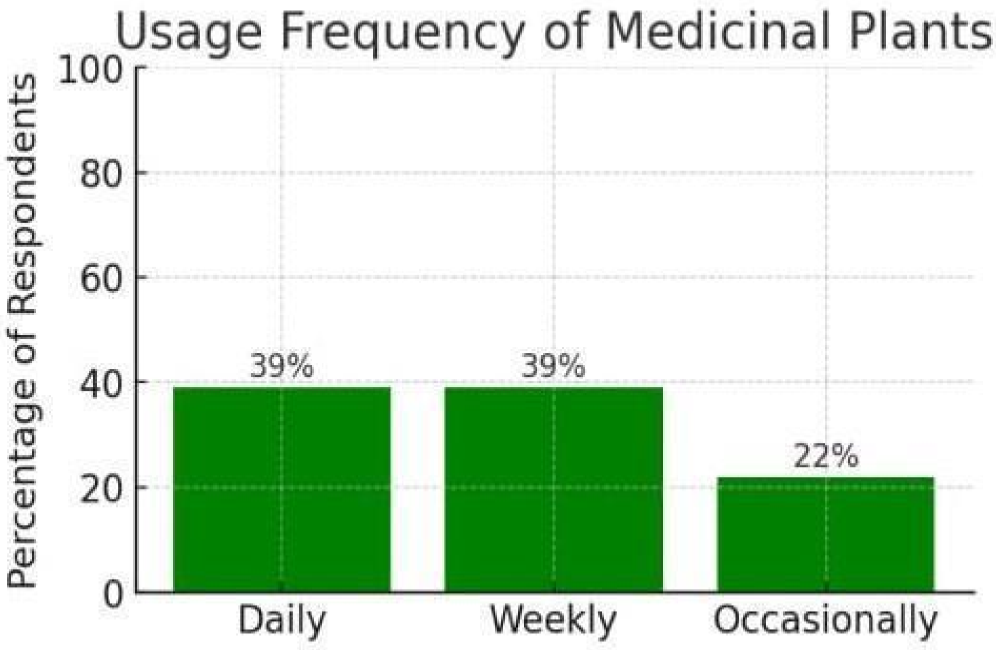
Table 2: Usage Frequency of Medicinal Plants

Frequency	Respondents	Percentage
Daily	78	39%
Weekly	78	39%
Occasionally	44	22%

Graph 1: Medicinal Vs Aromatic Plant Preference

(Graph would show 78% medicinal plant preference, 64% aromatic plant preference.)





Discussion

The survey results indicated that medicinal plants were more frequently used than aromatic plants, but the latter had significant demand in the wellness industry. Indian respondents favored tulsi, neem, and ashwagandha, while global respondents preferred lavender, peppermint, and ginseng. Farmers reported higher profitability from aromatic plant cultivation due to essential oil markets. Literature review findings supported these trends, with multiple studies showing rising essential oil exports from India. Challenges included lack of standardization, post-harvest losses, and global competition.

Conclusion

It was concluded that medicinal and aromatic plants represented a vital sector for healthcare and trade. India's biodiversity provided an advantage, but sustainable cultivation and global marketing strategies were essential. Collaboration between traditional knowledge holders, scientists, and policymakers was required to strengthen this sector.

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18. Dietary Acrylamide Exposure in Central Uttar Pradesh: A Risk Assessment of Processed Food Consumption among Adolescents.

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Abstract

This review is focused on the dietary habits of adolescents in the Central region of Uttar Pradesh, with a specific focus on the consumption of processed and fried foods, and the resulting exposure to acrylamide, a potential carcinogen. Acrylamide (C_3H_5NO) is a toxic chemical compound formed in starchy foods during high-temperature cooking processes such as frying, baking, and roasting, primarily through the Maillard reaction. Adolescence is a crucial period for establishing lifelong dietary patterns, and the increasing trend of consuming energy-dense, nutrient-poor foods like fast food and packaged snacks in India raises significant public health concerns. Various survey reports are explored the dietary preferences and food consumption frequency of adolescents. Results revealed that a high frequency of fast food consumption among the Adolescence, exceeding the levels of acrylamide (International food safety organizations). Thus this review explains acrylamide formation, its health risks and what can be done to reduce its presence in our food.

Keywords: Acrylamide, Dietary Habits, Adolescents, Junk Food, Uttar Pradesh, Public Health

1. Introduction

In 2002, Acrylamide was first discovered in the food items. It forms when starchy foods like potatoes or bread are cooked at high temperatures. Though found in small amounts, long-term exposure can be harmful. The concern is not only about individual health but also about how our cooking and eating habits affect the environment and society. Acrylamide is highly pertinent, sitting at the nexus of India's ongoing nutritional transition and the global concern over food-borne chemical contaminants [1]. In India, adolescents are play a pivotal role where lifelong dietary patterns are established, yet this stage is increasingly marked by the consumption of energy-dense, nutrient-poor, processed foods, raising significant alarm regarding non-communicable disease (NCD) risks in India [2, 3]. By the IARC, Acrylamide is a "probable human carcinogen" [4]. Its formation via the Maillard reaction in starchy foods cooked at high temperatures ($>120^{\circ}$C) means that popular processed snacks like potato chips, biscuits, and fried items are primary sources of exposure [5]. The study's focus on adolescents in Central UP—a region undergoing rapid urbanization and market penetration of packaged foods—is particularly justified, as children and adolescents exhibit higher exposure per unit of body weight compared to adults, magnifying the potential long-term risk [6, 7].

This review assesses the methodology and core findings described in the abstract against existing literature and food safety frameworks, highlighting the study's scientific and public health significance.

Fig.: A photo showing a group of diverse adolescents in Central Uttar Pradesh consuming popular processed snacks like potato chips and biscuits in a typical market or school-break setting.

Fig. : An image depicting a variety of common, locally available processed and fried food products (like packaged chips, different types of biscuits, samosas, French fries) arranged as if being collected for laboratory analysis.

Fig.: An image showing a local street food vendor in Central Uttar Pradesh preparing fried snacks (like samosas or pakoras) in a large kadhai (wok), emphasizing the high-temperature cooking methods.

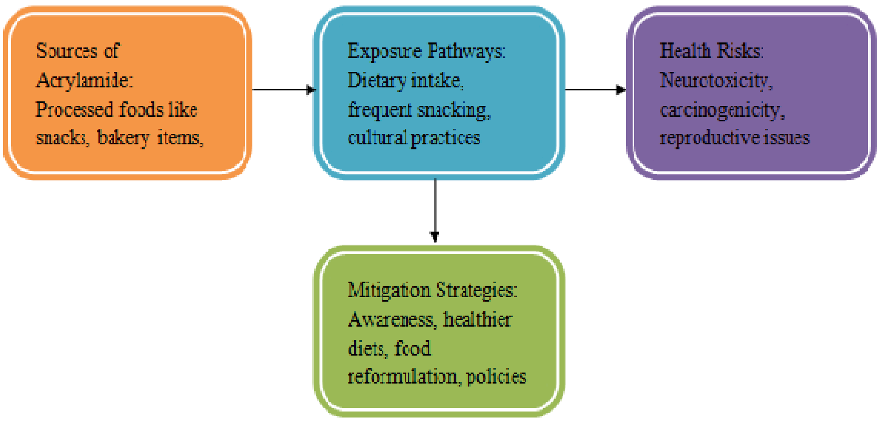
2. Critical Analysis of Methodology

2.1. Study Design and Relevance

The abstract confirms the use of a **cross-sectional survey combined with laboratory analysis**. This mixed-method approach is the standard, most effective design for a dietary exposure assessment [8]. The survey captures actual consumption patterns (frequency and preference, termed "junk food consumption"), while the chemical analysis provides the crucial concentration data (µg/kg) necessary for a quantitative risk calculation.

The selection of study food products—French fries, biscuits, and potato chips—is highly relevant to the Indian context, as these categories consistently emerge as major contributors to acrylamide intake globally and in Indian consumption studies [9, 10]. Studies have shown that even popular Indian snacks, particularly deep-fried varieties common in street food, often contain elevated levels of AA compared to international benchmarks due to preparation methods like high-temperature frying and oil reuse [11, 12].

A Framework of Acrylamide



2.2. Geographic Specificity

The study's focus on *Central Uttar Pradesh* addresses a significant geographical data gap. Most existing Indian data on dietary habits and contaminant levels are concentrated in major metros (e.g., Delhi, Kolkata, Southern provinces) [13]. Localized food production methods, vendor practices (e.g., oil type, frying duration), and regional food preferences can dramatically alter acrylamide content, making this regional data indispensable for local food safety regulation [14].

3. Review of Core Findings and Risk Implications

3.1. High Frequency of Junk Food Consumption

The finding of a **"high frequency of junk food consumption"** is in line with numerous reports indicating a strong shift toward ultra-processed food (UPF) intake among Indian adolescents, often at the expense of traditional, nutrient-rich foods [3, 15]. The abstract implies that this consumption is not merely occasional but significant enough to constitute a major exposure pathway.

3.2. Elevated Acrylamide Concentration in Food Products

A "significant proportion of the analyzed food products exceed the benchmark levels" is the most alarming data point. International bodies like the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have established indicative values for monitoring and mitigating acrylamide in food [16]. When local samples surpass these benchmarks, it directly signals a failure in local manufacturing or food preparation practices (especially in street-vended items) to implement widely known acrylamide mitigation strategies [17]. The actual risk assessment, likely utilizing the Margin of Exposure (MOE) approach is strongly suggested by the "substantial dietary acrylamide exposure." Given the genotoxic and carcinogenic nature of acrylamide, an MOE significantly below the cautionary threshold of 10,000 would validate the study's public health warning [18].

4. Public Health Significance and Recommendations

The study successfully bridges the gap between dietary behaviour and chemical risk, leading to robust and timely public health recommendations.

4.1. Regulatory Imperative

The call for **"regulatory monitoring"** is critical. In the absence of stringent, consistently enforced standards in India, especially at the state level (FSSAI/State FDA), manufacturers and vendors have little incentive to implement costly mitigation efforts. Specific, enforceable benchmark levels for common high-risk products like potato chips and biscuits are essential, as are training and auditing standards for local food vendors who prepare fried snacks [19].

4.2. Targeted Intervention

The recommendation for **"nutritional education programs"** must be targeted to the central finding: the *risk* in specific foods. Simply banning or taxing junk food is less effective than teaching adolescents and parents to identify the types of foods and preparation methods (e.g., dark brown frying) that are high in AA [20]. School-based interventions have proven crucial in modifying adolescent dietary habits in India [13, 15].

5. Conclusion

The present assessment highlights that adolescents in Central Uttar Pradesh are at a measurable risk of dietary acrylamide exposure due to frequent consumption of processed and fried foods such as potato-based snacks, bakery items, and packaged fast foods. Given the vulnerability of this age group, even moderate intake of acrylamide-containing foods may contribute to long-term health risks, including neurotoxicity, reproductive issues, and potential carcinogenic effects. The findings emphasize the urgent need for awareness campaigns targeting adolescents, parents, and food vendors to promote healthier dietary practices and reduce reliance on heavily processed products. Moreover, strengthening food safety regulations, encouraging reformulation strategies by manufacturers, and promoting traditional low-acrylamide cooking methods can significantly lower exposure levels.

Future Research Directions

To better understand and manage this emerging public health concern, future studies should focus on:

1. **Biomonitoring studies** using biomarkers (such as hemoglobin adducts of acrylamide) to quantify actual internal exposure among adolescents.
2. **Longitudinal epidemiological tracking** to establish the long-term health impacts of acrylamide exposure during adolescence.
3. **Food chemistry and processing research** to identify effective mitigation strategies that lower acrylamide formation without compromising taste and cultural food preferences.
4. **Behavioral and nutritional studies** examining adolescents' food choices, awareness levels, and responsiveness to educational interventions.
5. **Policy-based evaluations** to assess the effectiveness of national and regional food safety regulations in reducing dietary acrylamide exposure.

Overall, a multidisciplinary approach combining toxicology, nutrition, food science, and public health policy is essential to safeguard adolescent well-being and ensure a healthier future generation.

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19. Antioxidant Potential of *Murraya koenigii* Against Oxidative Damage: A Review

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Abstract

Oxidative stress underpins many chronic illnesses, diabetes, cardiovascular disease, cancer and neurodegeneration and plant-derived antioxidants are actively being investigated as safer, complementary strategies. *Murraya koenigii* (curry leaves) contains carbazole alkaloids, flavonoids, phenolic acids and volatile oils that together produce measurable antioxidant activity. This review synthesizes phytochemical, in vitro, in vivo and clinical evidence, emphasizing mechanisms (direct radical scavenging, induction of endogenous defenses via Nuclear Factor erythroid 2 related factor 2/Heme Oxygenase-1 [Nrf2/HO-1], and suppression of Nuclear Factor kappa B [NF-κB]) and contrasting *Murraya koenigii* with other widely studied botanicals. We identify key strengths (consistent preclinical data), major limitations (lack of extract standardization, poor bioavailability of key alkaloids, and sparse large-scale clinical trials) and practical research priorities (standardized extracts, pharmacokinetics, omics-driven mechanistic studies, and randomized controlled trials). The review closes with concrete translational recommendations for nutraceutical development.

Keywords: *Murraya koenigii*; curry leaves; antioxidants; oxidative stress; carbazole alkaloids; nutraceuticals

Introduction

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are normal by-products of aerobic metabolism that act as cellular signals at low concentrations but cause oxidative damage when produced excessively (Khan *et al*., 2021). Cells rely on enzymatic defenses such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and the tripeptide Glutathione (GSH) to maintain redox balance, and measurement of Malondialdehyde (MDA) or Total Antioxidant Capacity (TAC) are common ways to quantify oxidative burden in experiments (Bhat *et al*., 2018). When endogenous systems fail during chronic disease, dietary and botanical antioxidants can provide complementary protection. *Curry leaves*, from the species *Murraya koenigii* (family Rutaceae), are widely consumed in South Asian cooking and have a long history in traditional medical systems. Modern phytochemical and pharmacological studies show that *Murraya koenigii* is chemically distinct from many polyphenol-rich herbs because of its carbazole alkaloid content alongside flavonoids and phenolic acids (Sharma & Gupta, 2024). This combination suggests both immediate radical scavenging capacity and longer-term modulation of cellular antioxidant pathways, motivating a focused synthesis of current evidence.

Ethnopharmacology and Traditional Uses

Murraya koenigii features prominently in Ayurveda, Siddha and Unani systems. Classical texts and ethnobotanical surveys list its leaves for digestive complaints (carminative and appetite-stimulating uses), fever, wound healing, skin disorders, and as a hair tonic (Priyadarshini *et al*., 2022; Patel *et al*., 2021). In many rural traditions the leaves are used as household remedies for nausea, morning sickness and insect or snakebite management; concurrently, curry leaves are used daily as a culinary herb, which supports the idea of achievable, low-dose nutritional exposure. Importantly, several traditional claims now have experimental support. For example, animal studies showing hepatoprotection and blood-glucose modulation map plausibly to traditional claims of liver and metabolic benefits (Shah & Juvekar, 2015; Biswas & Roy, 2016). This translational continuity—traditional use → preclinical signal—strengthens the rationale for clinical evaluation of standardized curry-leaf preparations and for examining dietary use as a preventive strategy in populations at risk of oxidative-stress related disorders.

Figure 1. Whole Plant of *Murraya koenigii*

Phytochemistry

Among all parts of *Murraya koenigii*, the leaves have been most extensively studied due to their high concentrations of bioactive compounds, including carbazole alkaloids, flavonoids, and phenolic acids (Gupta & Sharma, 2015). The most distinctive constituents are carbazole alkaloids such as mahanimbine, girinimbine, and koenimbine which have been shown to modulate transcriptional antioxidant responses and enhance enzymatic defenses in cells (Bhat *et al.*, 2018). Flavonoids like quercetin and kaempferol, together with phenolic acids including gallic and caffeic acid, provide classical radical-scavenging and metal-chelating properties (Shah & Juvekar, 2015; Tiwari & Kumar, 2015). Volatile compounds, such as caryophyllene and linalool, contribute additional membrane-stabilizing and antimicrobial effects (Kumar & Singh, 2021).

Preclinical and Antioxidant Studies

Numerous in vitro and in vivo studies have investigated the antioxidant and protective effects of *Murraya koenigii* extracts. In vitro radical scavenging assays using methanolic leaf extract demonstrated strong free radical neutralization, with IC_{50} values ranging from 30 to 50 µg/mL, as determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Oxygen Radical Absorbance Capacity (ORAC) methods (Gupta & Sharma, 2015; Anand & Babu, 2017). Similarly, the ethanolic extract exhibited significant reducing power in Ferric Reducing

Antioxidant Power (FRAP) and Cupric Ion Reducing Antioxidant Capacity (CUPRAC) assays across concentrations of 50–400 µg/mL, with activity closely correlated to the flavonoid content of the extract (Anand & Babu, 2017). In vivo studies further support the antioxidant potential of curry leaf. In a carbon tetrachloride (CCl_4)-induced hepatotoxicity model in rats, administration of ethanolic leaf extract at 200 mg/kg restored liver enzyme activities, reduced malondialdehyde (MDA) levels, and improved histological architecture of the liver, indicating hepatoprotective effects (Shah & Juvekar, 2015). In streptozotocin (STZ)-induced diabetic rats, methanolic extract at 300 mg/kg significantly lowered blood glucose levels, enhanced catalase (CAT) and glutathione peroxidase (GPx) activities, and attenuated oxidative tissue damage (Biswas & Roy, 2016). Furthermore, neuroprotective effects of *M. koenigii* have been observed with alkaloid-enriched fractions in rodent models. Treatment improved cognitive performance and reduced reactive oxygen species (ROS) levels, demonstrating enhanced oxidative status and potential benefits against neurodegenerative conditions (Singh & Patel, 2021). Collectively, these studies highlight the potent antioxidant and protective properties of *Murraya koenigii*, which are attributed to its rich phytochemical profile, particularly flavonoids and carbazole alkaloids.

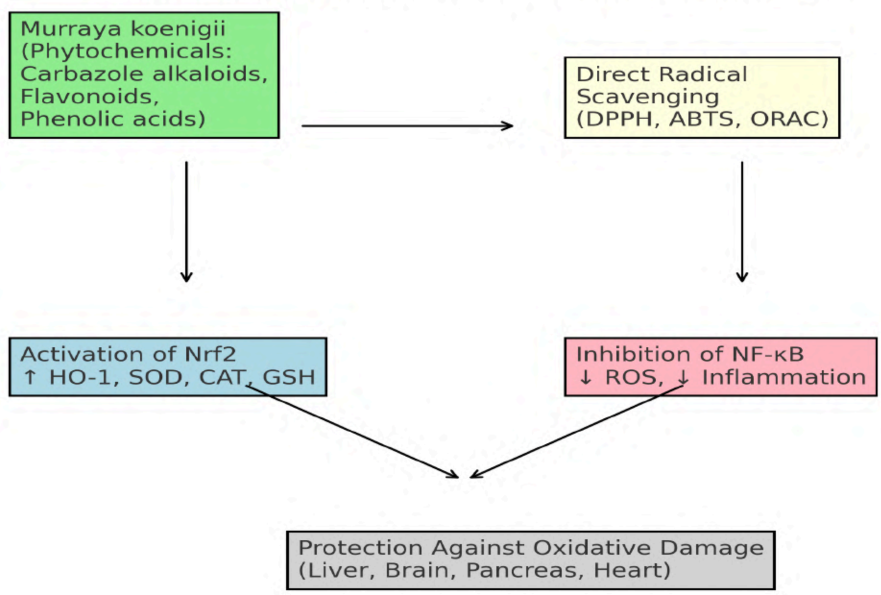

Figure 2. Schematic representation of antioxidant mechanisms of *M. koenigii*. Phytochemicals such as carbazole alkaloids, flavonoids, and phenolic acids contribute to direct radical scavenging (DPPH, ABTS, ORAC assays), activation of Nrf2 pathway

(upregulation of HO-1, SOD, CAT, GSH), and inhibition of NF-κB (reducing ROS and inflammation), leading to protection against oxidative damage in vital organs.

Clinical and Nutraceutical Studies of *Murraya koenigii*

Several clinical and nutraceutical studies have explored the therapeutic potential of *Murraya koenigii* (curry leaf), particularly in managing dyslipidemia, type 2 diabetes, and oxidative stress. In an open-label controlled study, dyslipidemic adults were administered 10 g/day of curry leaf powder for eight weeks. This intervention resulted in a significant reduction in serum triglycerides and total cholesterol, while simultaneously enhancing superoxide dismutase (SOD) activity, indicating improved antioxidant defense (Varghese & Mathew, 2019). In patients with mild type 2 diabetes, a randomized polyherbal trial assessed the effects of a capsule containing 500 mg/day of *Murraya koenigii* over 12 weeks. Participants showed a notable decrease in HbA1c levels, reflecting better glycemic control, along with increases in SOD and catalase (CAT) activities, highlighting the extract's capacity to mitigate oxidative stress associated with diabetes (Manoharan *et al.*, 2018). Furthermore, a safety and pharmacokinetic trial evaluated a 300 mg/day encapsulated extract of *M. koenigii* in healthy volunteers over a 12-week period. The study confirmed the extract's safety and observed a mild increase in antioxidant markers, suggesting potential health-promoting effects even in non-diseased individuals (Upadhyay & Verma, 2022).

These clinical and nutraceutical studies collectively support the use of *M. koenigii* as a natural intervention with multiple health benefits, including lipid-lowering, glycemic control, and enhancement of antioxidant status. Such findings provide a strong rationale for the inclusion of curry leaf in dietary supplements and functional foods aimed at promoting metabolic and oxidative health.

Discussion

The evidence indicates that *Murraya koenigii* possesses significant antioxidant potential with both preclinical and limited clinical support; compared with well-studied botanicals like *Curcuma longa* (turmeric), *Camellia sinensis* (green tea), *Rosmarinus officinalis* (rosemary), *Withania somnifera* (ashwagandha), and *Panax ginseng*, *Murraya koenigii* is unique due to its carbazole alkaloids (mahanimbine, girinimbine) combined with flavonoids and phenolic acids, which provide both direct radical scavenging and modulation of endogenous defense pathways (Nrf2/HO-1 activation and NF-κB inhibition); in vitro studies consistently show strong radical scavenging, in vivo models confirm hepatoprotective, antidiabetic, and neuroprotective effects, and preliminary human trials suggest improved lipid profiles and antioxidant enzyme activity; however, limitations include variable extract preparation, poor bioavailability of carbazole alkaloids, sparse clinical trials often confounded by polyherbal formulations, and lack of head-to-head comparative trials with other antioxidants; future research should focus on standardized extracts, pharmacokinetics, comparative efficacy trials, systems-biology approaches to understand broader redox signaling, and innovative delivery strategies such as nanoencapsulation or bioenhancer co-formulation, ultimately advancing *Murraya koenigii* from descriptive preclinical data to clinical validation.

Conclusion

Murraya koenigii exhibits notable antioxidant potential, attributable to its unique combination of carbazole alkaloids, flavonoids, and phenolic acids. Preclinical evidence consistently demonstrates its capacity to scavenge free radicals, enhance endogenous antioxidant enzymes, and modulate key signaling pathways such as Nrf2/HO-1 activation and NF-κB inhibition. Preliminary human studies suggest benefits for lipid regulation, glycemic control, and oxidative stress markers, although the data remain limited and underpowered. Compared with well-studied antioxidant plants like *Curcuma longa* (turmeric), *Camellia sinensis* (green tea), *Rosmarinus officinalis* (rosemary), *Withania somnifera* (ashwagandha), and *Panax ginseng*, *M. koenigii* offers a distinctive dual mechanism combining direct radical scavenging with transcriptional regulation of antioxidant defenses. Key limitations include variability in extract preparation, low bioavailability of carbazole alkaloids, and scarcity of standardized, large-scale clinical trials. Future research should prioritize chemically standardized extracts, pharmacokinetic profiling, dose-response human studies, comparative trials with other botanicals, and systems-level mechanistic analyses. Strategies to enhance bioavailability, such as nanoformulation or bioenhancer co-administration, may further optimize clinical efficacy. Overall, *Murraya koenigii* is a promising candidate for nutraceutical development and warrants rigorous clinical evaluation to translate preclinical insights into practical health applications.

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20. Herbivore-Induced Volatiles as Mediators of Tritrophic Interactions: Mechanisms and Applications in Plant Defence

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Abstract

Plants being immobile are always challenged by both the biotic and abiotic factors. The emission of volatile organic compounds (VOCs) is one of the advanced means of protection they employ. These VOCs are about 1 percent of the protective chemicals that are produced by plants and have also been found in over 1,700 flower species. They are used as internal signals, as communication between plants as well as influence plant interactions with other life forms in the environment. One group of these VOCs, called herbivore-induced plant volatiles (HIPVs) support an indirect defence system of plants. The HIPVs are able to attract predators and parasites that consume the herbivorous insects, confuse the insects, and even condition neighbouring plants to be better prepared against attacks. Synthesis of HIPVs is controlled by some specific chemical routes in the plant as jasmonic acid, ethylene and salicylic acid pathways triggered by insect feeding behaviour. As an example, mite attacks induce release of compounds such as ocimene, linalool, and methyl salicylate by avocado plants. The jasmonic acid pathway is found to increase the resistance of tomatoes to one particular mite species, *Polyphagotarsonemus latus*. HIPVs are diverse chemicals that include terpenoids, green leaf volatiles, jasmonates and phenolics. These chemicals have the ability to change insect behaviour and are involved in the complex relationships between plants, insects and the insect predators. The review focuses on the manufacture of HIPVs, the variety of chemical types that they include and the function that they serve in pest interactions between plants. It also discusses how they can be used in sustainable agriculture and new methods of controlling pests.

Keywords: *Tri-trophic interactions, Mites, Herbivore-induced plant volatiles, Plant defence, Secondary metabolites, Volatile organic compounds,*

1. Introduction

Plants are immovable and have established complex mechanisms to defend themselves against pests and diseases. Such a mechanism includes volatile organic compounds (VOC), which form approximately 1% of chemicals produced by plants. The VOCs are also essential in the defence of plants and in inter-plant communication (Jarmo & James, 2012). VOCs are used in inter-organism communication between herbivores, their predators (Kegge *et al*., 2013; Maffei, 2010; Niu, 2024). It depends on the herbivore releasing this or that VOC that will attract predators or not, and this will alter the dynamics of the system (Moayeri *et al*., 2007). As an example, citrus plants under attack by spider mites produce some VOCs that are HIPVs including linalool and undecyl cyclohexane and attract predatory mites. On the other hand, such compounds as pentadecimal reduce this attraction, and the chemical mixtures are specific (Song *et al*., 2024). The VOCs are very important in a number of ways. They are internal signals similar to hormones such as ethylene, methyl jasmonate and methyl salicylate. Also, they aid in the interaction between plants within and between the species. They are able to influence relationships at different levels in the food chain such as herbivores, pollinators, and natural enemies. As one example, jasmonic acid in tomatoes plants helps them to resist some mites (Grinberg *et al*., 2015). Specific VOCs can be preyed upon by specialized herbivores and their natural predators and repelled by generalist predators. The levels and types of HIPVs change in plants affected by mites, especially in the case of avocado plants infected with *Oligonychus yothersi*, especially when the mite populations are high and in summer (Erb *et al*., 2012; Heil, 2014). The changes are due to the increased production of some chemicals by the plant, such as jasmonic acid, ethylene, and salicylic acid. The result of these chemicals is the formation of terpenoids, green leaf volatiles, jasmonates and aromatic compounds that attract predators and parasitoids creating a three-level interaction (Maffei, 2010; Nagegowda, 2010; Takabayashi and Dicke, 1996; Yoneya *et al*., 2009; Penaflor *et al*., 2011). Even such compounds as indole could change the attractiveness of a plant to parasitoids. These HIPVs can be sensed by the olfactory receptors on herbivores antennae so that the predators can be able to detect the destroyed plants (Saveer *et al*., 2012). Moreover, HIPVs are capable of heightening resistance of adjacent plants by inducing defensive mechanisms such as production of extrafloral nectar in lima beans which is indirect protection. Current biotechnological solutions are set to strengthen the plant defenses with the objective of enhancing the production of VOCs by genetic engineering. They encompass methods of increasing HIPV production to either attract natural enemies or repel pests, semiochemicals, CRISPR-Cas genome editing, and gene drives, but with the consideration of the methods being safe in the natural environment (Taggar *et al*., 2024). The HIPVs have a big potential in the eco-friendly pest control by enhancing the indirect defense of plants and the overall health of the plants.

2. Plant Volatile Organic Compounds: Types and Biosynthesis

The volatile organic compounds (VOCs) that are released by plants represent a rich and varied type of chemical messages that are not only emitted by the plant but also between ecosystems. These substances control internal processes in plants, their communication with other plant species, and their interaction with other organisms, including herbivorous insects, pollinators, and natural predators of these insects (Jarmo & James, 2012; Niu, 2024). VOCs constitute circa 1 percent of all known secondary plant compounds and most of these have been detected in more than 1,700 plant families, which is indicative of their ecological and evolutionary importance. Recent studies (Hu, 2022) have shown that plant volatiles play a significant role in ecological communication and plant response to environmental stress, which is why they are essential in food chain interactions. The most often occurring, structurally diverse VOCs are terpenes which are synthesized on the basis of isoprene (C5H8) and are grouped according to the number of units: monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), tetraterpenes (C40), and polyterpenes (C5n). Several derivatives of terpenoids are also alcohols, aldehydes, ketones and esters. Chemical modifications like ring formation, oxidation, and rearrangement result in a variety of compounds such as a-pinene, limonene, linalool, and b-caryophyllene, many of which function as distinct signals in ecological communication (Maffei, 2010; Song *et al*., 2024). The terpenoid production is carried out in various regions of the plant cell. Isoprene and monoterpenes are produced by chloroplasts, which are usually associated with the heat tolerance and stress response of that plant. Plastids synthesize diterpenes and tetraterpenes via the methylerythritol phosphate (MEP) pathway, while the cytosol and endoplasmic reticulum (ER) generate sesquiterpenes and triterpenes through the mevalonate (MVA) pathway (Kegge *et al*., 2013). This process of specialization helps plants to continue with normal operations and also enable them to react speedily in case of danger. Genetic engineering of VOC processes illustrates the way in which altering these processes can increase the production of plant volatiles of certain species, which can help in crop protection (Taggar *et al*., 2024). Other important groups of VOCs are phenols and nitrogen or sulfur containing compounds. Singh *et al*. (2015) state that some phenolic VOCs such as methyl salicylate are also abundant when plants are infested by herbivores, especially aphids. The glucosinolates are sulfur rich compounds, which discourage the herbivores, and which are used as warning signals among plants, as well as their degradation products, including isothiocyanates (Halkier and Gershenzon, 2006). Recent results have shown that VOCs release in plants may differ depending on the type of herbivore and environmental stress, and it indicates their adaptive reactions (Perez-Hedo, 2024). Several pathways of plant hormones, particularly ethylene, jasmonic acid (JA), and salicylic acid (SA) also regulate the production of herbivore-induced plant volatiles (HIPVs). Such pathways allow plants to metabolically adjust quickly whenever herbivores attack. As an example, JA volatile methyl jasmonate helps tomatoes to synthesize proteins, which deter feeding and reproduction of the broad mite (*Polyphagotarsonemus latus*) (Grinberg *et al*., 2015). Recent results have also indicated that particular HIPVs like indole are also capable of modulating the attractiveness of herbivores to their natural predators and further reinforce ecological communication on varying levels of the food chain. The diversity of VOCs, the manner in which they are produced in various regions of the plant, and the influence of hormones on the regulation of VOCs production all demonstrate the significance of VOCs to plant defence and communication (Song *et al*., 2024; Taggar *et al*., 2024). Current studies are in progress to find viable solutions that can be used in sustainable measures to control pests using these compounds.

3. Herbivore-Induced Plant Volatiles (HIPVs)

Certain classes of volatile chemicals which plants emit when they are fed on by insects are known as herbivore-induced plant volatiles (HIPVs). HIPVs are not produced at all times as other chemicals; they are produced in a certain manner according to the type of insect that is feeding and the environment. These volatiles are one of the most ecologically important indirect defence mechanisms of plants acting as airborne cues which lower the level of herbivore pressure and improve the survival of plants. When herbivores begin feeding, plants typically exhibit two distinct volatile responses. The first is an immediate response, where stored compounds are rapidly released upon tissue damage. These often include green leaf volatiles (GLVs), which are emitted within seconds to minutes of damage and may directly deter herbivores or attract natural enemies (Jarmo & James, 2012). The second response is delayed and involves the de novo synthesis of volatiles triggered by herbivore-associated cues, such as oral secretions. This process, which unfolds over hours to days, is tightly regulated by hormonal pathways, particularly jasmonic acid (JA), salicylic acid (SA), and ethylene signalling.

HIPVs often overlap with constitutively emitted volatiles, but herbivore feeding can also elicit entirely new compounds. For example, *Phaseolus lunatus,* lima bean plants release only α-pinene and limonene; however, after 48 hours of spider mite feeding, they emit seven additional compounds, including DMNT *i.e.* (E)-4,8-dimethyl-1,3,7-nonatriene (Heil, 2014). Such inducible compounds provide reliable indicators of herbivory and increase the specificity of plant defence responses. Another well-studied HIPV, methyl salicylate, is synthesized from salicylic acid and plays a crucial role in signalling during aphid infestation.

Collectively, HIPVs act as a chemical "cry for help" by attracting predators and parasitoids of herbivores, thereby enhancing plant protection (Takabayashi & Dicke, 1996). In addition to recruiting natural enemies, HIPVs can repel herbivores, interfere with host location, or prime neighbouring plants to activate their defences. This ecological adaptability emphasizes their importance in plant–herbivore–predator (tritrophic) interactions and noteworthy for applications in sustainable pest management.

4. Triple Control Tri-Trophic Interactions and Ecological Functions of HIPVs.

The volatiles induced in plants by herbivores (HIPVs) are key facilitators in the tritrophic interactions existing between host plants, feeding herbivores and even the natural enemies that control the herbivore populations. These signals show an important role in indirect plant defence when acting in a erudite way as the plant is able to transmission the presence of herbivorous invasions, recruiting predators and parasitoids that reduce the population of the pests. This is the mechanism where the plants protect themselves without causing any direct harm to the herbivores and this is just a good example of an adaptive, energy-saving mechanism of pest control (Takabayashi and Dicke, 1996).

4.1 Indirect Plant Defence

HIPVs work similarly to chemical beacons in terms of guiding the natural enemies to the locations of the herbivore feeding. To give an example, the predatory mites belonging to the species of *Phytoseiulus persimilis* have a significant attraction to the HIPVs released by plants of lima bean (*Phaseolus lunatus*) then attacked by spider mites (*Tetranychus urticae*) (Dicke *et al*., 1993). Similarly, parasitoid insects like HIPV-using cues are used by the parasite to find cue, e.g., HIPV-emitting brasseries (Shiojiri *et al*., 2002). These interactions represent the contribution of plant volatiles very graphically to the stimulation of interspecific assistance in plant defence networks.

4.2 Multi-Herbivore Effects

As more than one plant is attacked by several species of herbivores, the outcome bouquet of HIPVs is more complex. This complexity may increase the recruitment of predators as well as interfere proximate to the bait-tactic effects on the efficiency of predation. In another study, the infestation of plants by a species that was already weakened by the presence of *Tetranychus urticae* caused the reduction of localization of predators by the *Oligonychus ilicis* (Marcos *et al*., 2010). In an opposite case, the co-occurrence of the *Spodoptera exigua* and *Tetranychus urticae* on lima beans enhanced the attraction of predators but by consequence, a decrease in the specific volatile emissions of cucumber (*Cucumis sativus*) (Boer et al., 2008). These results forward the idea of conditionality of plant responses to various herbivores, depending on the species of the plants and ecological setting.

4.3 Herbivore Behaviour and Interaction with other species

HIPVs too have a lot of influence on the behaviour of the herbivores. As an example, female parasites of tobacco (*Heliothis virescens*) moths prefer to shun tobacco plants (Nicotiana tabacum) that produce tobacco HIPVs after being attacked by moths (de Moraes *et al*, 2001). Aphids also change their preferences in regards to settlement in relation to compounds like methyl salicylate. Further, emitted HIPVs may vary dynamically depending on the identity and the concentration of herbivores attacking them; when HIPVs repel conspecifics, neighbouring intact plants may be the beneficiaries of a phenomenon known as associational resistance.

4.4 Case Studies of HIPVs Established in Crops

HIPVs have been documented across a spectrum of agricultural crops. When tomatoes are subjected to the jasmonic acid (JA) pathway, it triggers the production of volatile HIPVs which make the plants less appealing to the broad mite (*Polyphagotarsonemus latus*) compared to genotypes that were subjected to non-functional controls of the JA pathway (Grinberg et al., 2015). The HIPV profile of Avocado foliage attacked by the *Oligonychus yothersi* is changed, with higher levels of methyl salicylate, farnesyl cyanide, linalool, (Z)-ocimene and farnesene, thus attracting the predatory beetles, including *Parastethorus histrio* and *Oligotapygmaea* (Rioja et al., 2015). Volatile release by coconut palm infested with is a attractant to predatory mites such as the *Neoseiulus baraki* and *Proctolaelaps bickleyi*, but the latter prefer infested leaves whereas the former is focused on infested fruits (Ishiwari *et al*., 2007) (infested by *Aceria guerroenis*). The totality of these studies shows that HIPVs are very fundamental in supporting natural pest suppression in cultivated systems.

4.4.1 Tomato (*Solanum lycopersical*)

The tomato cultivars use the JA pathway to reduce herbivory by the broad mite (*P. latus*). A volatile form (Methyl jasmonate) of JA causes the foliage to accumulate proteinase inhibitors. Comparative studies of JA-deficient mutants (e.g., def-1) have been performed against wild-type plants and a distinct preference of the mites has been noted towards the latter with higher feeding preferences, oviposition and larval development observed in the mutant lines (Grinberg *et al*., 2015). These results underscore the central role of the HIPV-mediated JA signalling in decreasing the suitability of the niche by herbivores.

4.4.2 Avocado (*Persea americana*)

The mite infection rate in avocadoes triggers high levels of HIPVs, such as the methyl salicylate, farnesyl cyanide, linalool, (Z)-ocimene, (E)-ocimene, and farnesene. These emissions help host assemblages of natural enemies, especially the beetles, living epiphytically on the mite, such as, though not all, *Parastethorus histrio, Oligotapygmaea*. The concurrent action of ethylene, JA, and salicylic acid signals contributes to further intensification of volatile production especially in situations where there is an increased pest pressure (Rioja *et al*., 2015).

4.4.3 Coconut (*Cocos nucifera*)

The coconut palm which has been damaged by the *Aceria guerreronis* parasite synthesizes specific HIPV in both the foliar and floral tissues. The predator mite species, *Neoseiulus baraki* and *Proctolaelaps bickleyi* react differently, with the later exhibiting a distinct attraction in infested leaves and the former attracted to the scent of infested fruits (Ishiwari *et al*., 2007). This differential conscription emphasizes the malleability of plant volatile signalling to optimize interactions with the most effective natural rivals. Such studies together validate the fact that HIPVs are context-specific signals and dynamic and hence, coordination of an intricate ecological action amid the various plant-herbivore associations and hence, ascertaining the makeup of natural and cultivated ecologies.

5. Role of Secondary Metabolites in Plant Defence

Secondary metabolites play a major role in defences of a flora of plants. These substances play an important role not only in direct repelling action of the herbivores, but also in the indirect attracting of the predators by exerting chemicals under the condition of which other biological processes are carried out.

5.1 Terpenes

The most common and most varied type of secondary metabolites are terpenes that help in the production of the herbivore-induced plant volatiles (HIPVs). Their action can be categorized into two main segments: one, some of the terpenes have acute toxicity against herbivores, interruption of cellular constructions, or indiscipline of the digestive system; second, some procure terpenes can be semiochemicals that advertise the attaining creatures, including predators and parasitic wasps. Volatile terpenes such as beta, caryophyllene and linalool tend to be liberated during attack by an herbivore and led to tiaras pattern (Dudareva *et al*., 2013). Terpenes play a pivotal role in plant defense and ecology since they perform both a defensive and communicative role.

5.2 Phenolic

Phenolic, which are a second major group of defensive secondaries, metabolites represent a major group. The antioxidant activity helps them to prevent the plant against oxidative damage, and the ultraviolet-absorbing properties provide natural protection against the harmful UV rays similar to sunscreens. Increased crusts of phenols make the tissues as well uneatable or inedible by herbivores, curbing feeding hypotheses. The release of volatile phenolics like methyl salicylate that is formed as a result of salicylic acid plays an important role in herbivore attack when released and serves as a major signal in HIPV communication. Some other important functions of phenolics are direct antifeedant properties: in other words, salicylates were found to reduce the development of mites, slow syndrome progression, and misbalance their health condition (Singh *et al*., 2015).

5.3 Glucosinolates

Glucosinolates, which are typical Brassicaceae secondary metabolites, are broken down when the tissue is damaged as biologically active substances with isothiocyanates. Under these conditions, Halkier and Gershenzon (2006) show that these degradation products serve as an early warning system to the neighbouring plants, and at the same time cause them to be less palatable to insect herbivores. The counter one insect includes the cabbage aphid (*Brevicoryne brassicae*) which deposits the glucosinolates and then metabolizes it to isothiocyanates when attacked and, therefore, evades predation (Kazana *et al*., 2007).

These observations highlight the interactive co-evolutionary arms race between plants and herbivores through chemical defence. The acrylonitrile compound is a complex comprising of terpenes, phenolics and glucosinolates and have been shown to have a complex defence system that integrates multiple different defence mechanisms that include direct toxicity, aggressive behaviour, and attracting predators with volatile organophosphine emission These findings attest to the strategic utilisation of chemical compounds by plants in order to maximize their defence.

Table 1. Illustrations of herbivore induced plant volatiles (HIPVs), their chemical composition and use in the environment.

Compound / Group	Chemical class	Induced by (Herbivore / Stress)	Plant system studied	Ecological function	Reference
(E)-DMNT	Homoterpene	*T. urticae* (spider mite)	Lima bean (*Phaseolus lunatus*)	Attracts predatory mite *P. persimilis*	Dicke *et al*., 1993
TMTT	Homoterpene	Caterpillar feeding	Maize, tobacco	Attracts parasitoids	Heil, 2014
Methyl salicylate (MeSA)	Phenolic derivative	Aphids, *O. yothersi*	Grapevine, avocado	Attracts predators, repels herbivores	Ozawa *et al*., 2012
β-ocimene	Monoterpene	*T. urticae*, *O. yothersi*	Lima bean, avocado	Enhances predator attraction	Horiuchi *et al*., 2003; Rioja *et al*., 2015
α-farnesene	Sesquiterpene	Spider mites	Tea, cucumber	Alters predator search behaviour	Ishiwari *et al*., 2007
Isothiocyanates	Glucosinolate products	Aphid feeding/ damage	Brassicaceae	Act as alarm pheromones to conspecifics	Kazana *et al*., 2007
α-pinene, 3-carene, α-/β-phellandrene, β-ocimene	Monoterpenes	Pest presence (*T. absoluta*)	Tomato	Predator recruitment via kairomones (*N. tenuis*)	Messina *et al*., 2023
α-pinene, β-phellandrene, caryophyllene	Monoterpenes /Sesquiterpene	Flood + herbivory	Tomato	Enhanced HIPV emission under multiple stress	Zhang *et al*., 2022
Altered VOC blend (reduced emissions)	Terpenes, phenolics	AMF colonization + herbivory	Tomato	Growth-defense trade-off	Sbrana *et al*., 2023
VOCs affecting detox response	Mixed HIPVs	Pest exposure (*S. litura*)	Tomato	Induced detox enzyme & gene expression in pest	Wei *et al*., 2022

Altered HIPVs enhancing predator attraction	Multiple VOCs	Soil fungi symbiosis + herbivory	Tomato	Microbe-mediated indirect defense	Guerrieri *et al.*, 2022
HIPV delay signalling	Volatiles	Herbivory (neighboring cotton plants)	Cotton	Temporal specificity in interplant signalling	Li *et al.*, 2023
Terpenes, DMDS	VOCs (terpenes, sulfur compounds)	*S. exigua* feeding	Wild vs. domesticated tomato roots	Evolutionary divergence in root defense	Ximénez-Embún *et al.*, 2022

6. HIPVs are influenced by environmental factors.

All the time, the secretion of herbivore-induced plant volatiles (HIPVs) does not occur in the same manner. It varies according to the surroundings and the well-being of the plant. This flexibility means that plants adapt their defences to the things happening around them, but complicates trying to work out the role of these volatiles in natural environments.

6.1 Light

The amount of light that an individual plant receives is one of the primary factors that influence the degree of HIPVs it releases. During the day, the amount of these volatiles produced by plants is greater than at night. This is related to the photosynthesis process since the materials required to produce the volatiles are produced by the plant producing food. In addition, the plant produces more terpenoids when it has lighter and these are produced in chloroplasts. This enhances the capacity of the plant to protect itself against the insects and their predators at their peak times.

6.2 Temperature

The temperature is significant in the number and types of HIPVs discharged by a plant. As an illustration, release of significant volatiles such as (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and methyl salicylate (MeSA) is peak at 30degC but decreases both under lower and higher temperatures in lima beans. That is, the plant can only operate optimally within a range of temperature. At temperatures that are not within that range, such factors as cell membrane fluidity or enzyme activity may be compromised. This may decrease the quantity of volatile emitted and lower the defence capacity of the plant to withstand adverse conditions.

6.3 Nutrient Availability

The availability of nutrients in the soil also changes how plants respond with HIPVs. In a case, the predatory mites that lack vitamin A are more inclined to diverse types of HIPVs. This shows that the ability of predators to react to plant volatile might change throughout their health. In a broader sense, the characteristics and amount of HIPVs produced by a plant is dictated by the nutrients supplied to the same. These examples suggest that the modes of communication of plants via HIPVs are very dependent on the surrounding. Effects of HIPVs are determined by light, temperature and nutrients interaction. Such factors are especially important to realize when one thinks about how the environment, e.g. climatic change, can contribute to interactions between plants and animals.

7. Application and Future Opportunities

HIPVs studies have been very promising in the fields of agriculture, biotechnology and conservation. HIPVs also can offer a new avenue of reducing pesticide use which is a natural signalling molecule that can be deployed to protect crops and environment.

7.1 Pest Control in Agriculture

A large application of HIPVs are work as an integrated pest management (IPM). HIPV can be designed as synthetic products and can be deployed in agricultural fields and thus, attract natural predators of pests, thus, controlling their population. As an example, in vineyards the application of dispensers releasing methyl salicylate was used to increase the number of predators and decreasing the problem of spider mites. Unlike chemical pesticides, which often mess up natural balance, HIPV-based methods support existing food chains and help control pests in a more sustainable way. A new biotechnology innovation has been developed by the company named evolutionary cuisine.

7.2 New Biotechnology Innovations

The company has created a new innovation in biotechnology called evolutionary cuisine. The new opportunities provided by the plant biotechnology have led to using HIPVs as a natural defence system. The breeding practices and genetic engineering have the potential to alter the way plants produce HIPVs so that they produce more or different types of volatiles involved in defence. As an illustration, the enhancement of the pathways that lead to jasmonic acid-regulated volatiles can be used to enable plants to be able to resist mites and caterpillars without compromising its crop yield. These innovations can provide a long-term alternative to the use of external chemicals and the plant itself will be less prone to attacks.

7.3. Conservation and Ecosystem Services

HIPVs have also been found to be significant in facilitating biodiversity by means of complicate interactions between various levels of the food chain. They also affect predator-prey interactions and the plant-plant communication. The study of these networks has the potential to aid conservation particularly in the management of habitats by the natural predators that have a role in the regulating of the populations of pests. Consequently, HIPVs do not only assist in farming, but also maintain natural ecosystems.

8. Future Directions

In spite of much work done in the laboratory, much remains to be known in bringing the HIPV knowledge into practice in the real life. Complexity in the field conditions is a factor as the light, temperature, and other herbivores may be present, making the use of HIPVs more difficult. Future studies ought to be on: Determining how the production of HIPV is regulated through hormonal and molecular means. The production or the modification of crops to produce the optimal combination of HIPVs. Development of low-cost methods of field delivery of synthetic HIPVs. Examining how the volatile interactions change over time in relation to the environment. It will have HIPVs to assist us in finding new ways of cultivating the farm in a more sustainable manner. With the cooperation of technology, ecological knowledge will decrease the use of chemical pesticides, biodiversity support and friendlier farming systems.

9. Conclusion

Herbivore-induced plant volatiles (HIPVs) are multifaceted, complex defences, which increase the likelihood of a plant to survive by improving attraction of natural predators and simultaneously repelling pests. Experiments on tomatoes, lima beans, avocadoes and cocoanuts indicate that HIPVs exhibit a wide range of crops and pests to which they are sprayed. To come up with effective, long-term solutions to pest management, the HIPVs should be known in their chemistry, how they would work in the environment, and how they can be implemented. All HIPVs together create a smart evolution mechanism that connects ecological webs and helps the plants to defend themselves. The recent improvement of HIPVs can be significant in changing agriculture in to a more sustainable practice in the future.

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21. Health and Disease Management in Animal Science

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Abstract

Health and disease management in animal science is a crucial pillar of sustainable agriculture, public health, and food security. This paper explores traditional and modern practices in disease prevention, diagnosis, and treatment in livestock and companion animals. It emphasizes the importance of integrated health management systems, including biosecurity, vaccination, nutritional strategies, emerging technologies, and policy frameworks. With the rise in zoonotic diseases and antimicrobial resistance, a holistic One Health approach is advocated. The research concludes by highlighting challenges and opportunities in building a resilient animal healthcare ecosystem.

Keywords: Animal health, zoonotic diseases, biosecurity, vaccination, One Health, antimicrobial resistance, disease surveillance, livestock management, veterinary technology

Introduction

Animal health is not only essential for the productivity and welfare of livestock but also for the overall health of ecosystems and human populations. Disease outbreaks in animals can devastate economies, disrupt food chains, and trigger public health emergencies, as seen with avian influenza, swine flu, and bovine spongiform encephalopathy. In this context, effective health and disease management in animal science requires an integrative approach blending traditional veterinary care with advanced technologies and public health strategies.

Importance of Disease Management in Animal Science

The management of animal diseases directly influences

i. ***Food security***: Healthy animals produce safe, quality meat, milk, and eggs.

ii. ***Economic sustainability***: Disease outbreaks cause financial loss to farmers and industries.
iii. ***Public health***: Many animal diseases are zoonotic, transmitting to humans.
iv. ***Animal welfare***: Ethical treatment and health monitoring are necessary for responsible animal husbandry.

Types of Animal Diseases

Animal diseases are broadly classified into:

Infectious diseases: Caused by bacteria (e.g., brucellosis), viruses (e.g., rabies), parasites (e.g., trypanosomiasis), and fungi.

Non-infectious diseases: Include nutritional deficiencies (e.g., milk fever), genetic disorders, and environmental illnesses (e.g., heat stress).

Zoonotic diseases: Transmissible from animals to humans, such as anthrax, leptospirosis, and tuberculosis.

Preventive Measures and Biosecurity

Biosecurity is the cornerstone of disease prevention in animal science. measures include:

- Controlled farm access
- Quarantine of new or sick animals
- Sanitation of equipment and housing
- Vector control (ticks, mosquitoes)
- Proper carcass disposal
- Vaccination is another powerful preventive strategy. For instance, foot-and-mouth disease (FMD) vaccination programs have significantly reduced outbreaks in Asia and South America.

Diagnostic Tools and Surveillance

Early diagnosis and disease surveillance are vital for controlling outbreaks. Modern tools include:

- Molecular diagnostics (PCR, ELISA) for rapid pathogen detection

- Mobile veterinary apps for field diagnostics
- Geographic Information Systems (GIS) for disease tracking
- Wearable sensors and biosensors for real-time health monitoring
- Surveillance systems, such as the World Organisation for Animal Health (WOAH) and India's NADRS (National Animal Disease Reporting System), play a key role in reporting and managing disease events.

Nutritional and Environmental Management

Balanced nutrition strengthens immunity in animals and prevents diseases like rickets, ketosis, or anemia. Farmers are encouraged to:

- ✔ Ensure species-appropriate feed
- ✔ Provide mineral and vitamin supplements
- ✔ Maintain clean and ventilated housing
- ✔ Offer proper hydration and reduce stressors
- ✔ Environmental enrichment is especially critical for poultry and pigs to reduce abnormal behaviors and promote natural immunity.

Antimicrobial Resistance and Its Management

The misuse of antibiotics in animal farming has led to alarming rates of antimicrobial resistance (AMR), posing a serious threat to human and animal health. To combat this:

- ✔ Antibiotics should be used only under veterinary guidance
- ✔ Growth-promoting antibiotics must be restricted
- ✔ Alternatives such as probiotics, herbal medicine, and phage therapy are gaining popularity

Global initiatives like the WHO's Global Action Plan on AMR urge coordinated efforts between nations, especially in livestock-dense countries.

Role of Emerging Technologies

The future of animal disease management lies in innovation:

- Artificial Intelligence (AI): Predict disease outbreaks through big data.
- Genomics and CRISPR: Enable breeding of disease-resistant animals.

- Drones and IoT: Monitor remote herds and detect anomalies.
- Blockchain: Ensure traceability in meat and dairy products.

For example, precision livestock farming (PLF) in the EU uses smart collars and automated health alerts to enhance dairy cow management.

The One Health Framework

The One Health approach recognizes the interconnectedness of human, animal, and environmental health. It emphasizes:

- Multisectoral collaboration (veterinarians, medics, ecologists)
- Integrated surveillance systems
- Joint training and policy-making

India's "One Health India" initiative, launched in 2021, is a step forward in coordinating disease management between animal and human health sectors.

Challenges in Animal Disease Management

Despite progress, several hurdles remain:

- ➔ Shortage of skilled veterinary personnel
- ➔ Low farmer awareness and education
- ➔ Weak cold-chain infrastructure for vaccines
- ➔ Budget constraints and underreporting of diseases
- ➔ Climate change is also altering disease patterns, with vector-borne diseases like bluetongue emerging in new regions.

Recommendations

a. Strengthen veterinary infrastructure, especially in rural and tribal areas
b. Promote farmer training in early disease recognition and hygiene
c. Encourage interdisciplinary research on zoonoses and AMR
d. Subsidize vaccination and diagnostic services for small-scale farmers
e. Leverage public-private partnerships to scale health technology adoption

Conclusion

Effective health and disease management in animal science is a linchpin of sustainable agriculture, food safety, and public well-being. By embracing biosecurity, modern diagnostics, vaccination, and a One Health approach, we can build resilient animal health systems. Governments, scientists, farmers, and global health bodies must work together to ensure healthier animals, safer food, and a better future.

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