ROGARAKSHAK: INTEGRATED STRATEGIES FOR CROP PROTECTION

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Rogarakshak: Integrated Strategies for Crop Protection

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PREFACE

Agriculture remains the backbone of food security and rural livelihoods, yet it faces persistent challenges from pests, diseases, and weeds that significantly reduce crop productivity and quality. In this context, Rogarakshak: Integrated Strategies for Crop Protection is envisioned as a comprehensive and practical guide that emphasizes a holistic approach to safeguarding crops while promoting sustainable agricultural practices.

The term Rogarakshak—meaning "protector from diseases"—aptly reflects the core objective of this work: to equip learners, practitioners, and stakeholders with integrated knowledge and strategies for effective crop protection. This book brings together the principles of Integrated Pest Management (IPM), disease management, weed control, biological control, and the judicious use of chemical measures, highlighting their complementary roles in achieving eco-friendly and economically viable farming systems.

This preface sets the tone for an interdisciplinary understanding of crop protection by blending traditional wisdom with modern scientific advancements. Special emphasis is placed on preventive measures, early diagnosis of crop disorders, and decision-making based on field-level observations, weather patterns, and technological tools. The integrated strategies discussed herein aim not only to reduce crop losses but also to minimize environmental impact, preserve biodiversity, and ensure the safety of farmers and consumers.

Rogarakshak: Integrated Strategies for Crop Protection is designed to serve as a valuable resource for students of agriculture, extension workers, researchers, and progressive farmers. It aspires to foster awareness, innovation, and responsible practices that contribute to resilient agricultural systems and sustainable food production. Through this work, we hope to inspire a proactive and informed approach to protecting crops—ensuring healthier harvests today and a secure agricultural future for generations to come.

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Dr.T.Meera, has completed Under-graduation, Post-graduation and Doctor of Philosophy (Plant Pathology) at Faculty of Agriculture, Annamalai University, Tamil Nadu, in 2013. She has experience of ten years in teaching, research, and outreach extension activities and she is presently working as an Assistant Professor (Plant Pathology), School of Agriculture, VISTAS. She received the funded government project and the Best Oral Presentation award in the International Conference. She published 24 research articles, four review articles, five popular articles, 3 books, and 15 book chapters for double peer reviewed national and internationally reputed journals. She participated and presented the papers in four national and five international conferences and seminars in various states of India. She attended and organized various workshops and trainings from all over India. She is a member of four subject-related National Societies of India. She has overseen numerous experiential learning courses and assisted 5 undergraduate students with their project works**.**

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CHAPTER 1

INTRODUCTION TO INTEGRATED DISEASE MANAGEMENT HISTORICAL PERSPECTIVE AND CURRENT TRENDS

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Abstract

Integrated disease management (IDM) is a holistic approach that combines multiple strategies to control plant diseases, aiming for sustainable and environmentally friendly solutions. Historically, IDM emerged from the necessity to address the limitations and adverse effects of single-methods disease control such as chemical pesticides. Early practices focused on crop rotation, resistant varieties and cultural methods. The green revolution of the mid-20th century while boosting agricultural productivity also highlighted the need for integrated approaches due to the negative environmental impacts and resistance issues associated with chemical reliance. In recent decades, IDM has evolved to incorporate advances in biological control, genetic engineering and precision agriculture. Current trends emphasize the integration of cutting-edge technologies like sensing, big data analytics and artificial intelligence to monitor and predict disease outbreaks more accurately. Additionally, there is a growing focus on microbial consortia and natural predators as biological control agents alongside the development of disease-resistant crop varieties through genetic modification and CRISPR technology. Sustainable agriculture and climate change adaptation have also become central themes, promoting practices that reduce the carbon footprint and enhance ecosystem resilience. Overall, IDM represents a dynamic and evolving field that continues to integrate traditional knowledge with modern innovations to ensure sustainable plant health management.

Keywords: CRISPR, crop rotation, genetic engineering, climate change, biological control.

Introduction

Integrated disease management represents a paradigm shift in agricultural practices, addressing the limitations of single-method disease control by

employing a multifaced approach. Historically, plant disease management relied heavily on chemical pesticides which while initially effectively led to several unintended consequences. These included environmental degradation, non-target species harm, pesticides resistance and human health concerns. The roots of IDM can be tracked back to traditional agricultural practices such as crop, rotation, intercropping and the use of resistant plant varieties [1]. These methods grounded in ecological principles aimed to reduce the incidence and severity of diseases by creating less favourable conditions for pathogens. The advent of the Green Revolution in the mid-20th century, characterized by the widespread adoption of high-yielding crop varieties and synthetic agrochemicals brought significant increase in agricultural production. However, it also underscored the need for integrated approaches due to the negative impacts associated with heavy chemical use [2].

IDM began to integrate biological control methods, utilizing natural predators, parasites and microbial antagonists to suppress disease-causing organism. Advances in genetic engineering further enriched IDM, enabling the development of crops with enhanced resistance to diseases [3]. Precision agriculture technologies including remote sensing, big data analytics and artificial intelligence are revolutionizing IDM by allowing for real-time disease monitoring and predictive analytics. Current trends in IDM emphasize sustainability and adaptability to climate change. As the climate shifts new disease challenges emerge necessitating resilient agricultural practices [4]. Sustainable agriculture practices, which minimize the carbon footprint and promote ecosystem health, are integral to IDM strategies. IDM is a dynamic and evolving field that combines traditional knowledge with modern innovations. It aims to provide effective, sustainable and environmentally friendly solutions to plant disease management ensuring agricultural productivity and ecological balance for future generations [5].

Historical perspective of disease management

Early practices in Plant Disease Control

The history of plant disease management is rich with traditional practices that laid the groundwork for modern IDM. Early agricultural societies employed methods such as crop rotation, intercropping and the use of disease-resistant varieties to minimize the impact of plant diseases. These practices, grounded in empirical observations and local knowledge aimed to disrupt the life cycles of pathogens and reduce the incidence of disease [6].

The impact of Green Revolution

The mid-20th century Green Revolution marked as significant turning point in agricultural history, characterized by the widespread adoption of high-yielding crop varieties and synthetic agrochemicals. While this revolution significantly boosted global food production, it also exposed the limitations and drawbacks of heavy chemical reliance. Issues such as pesticide resistance, environmental pollution and health concerns prompted a re-evaluation of disease management strategies [7].

Emergence of integrated approaches

In response to these challenges, the concept of IDM began to take shape in the latter half of the 20th century. The realisation that no single method could effectively manage plant diseases in a sustainable manner led to the development of integrated approaches. These early IDM frameworks combined chemical, biological and cultural methods laying the foundation for more sophisticated and comprehensive strategies in subsequent decades [8].

Core components of IDM

Biological control methods

One of the key components of IDM is biological control, which involves the use of natural enemies to supress disease-causing organisms. This approach can include the introduction or augmentation of predators, parasites and microbial antagonists. Beneficial fungi and bacteria can be applied to crops to outcompete or directly inhibit pathogens [9].

Natural predators and parasites

Natural predators such as certain insects' species can be utilized to control populations of disease vectors. Parasitoids which lay their eggs in or on other insects, can also be effective in reducing pest populations that contribute to disease spread [10].

Microbial antagonists

Microbial antagonists including bacteria and fungi can inhibit pathogen growth through mechanisms such as competition, antibiosis and parasitism. *Trichoderma* species are known for their ability to control soil-borne pathogens through competitive exclusion and the production of antifungal compounds [11].

Genetic Engineering in Crop Disease Resistance

Advances in genetic engineering have significantly enriched IDM by enabling the development of crops with enhanced resistance to diseases.

Techniques such as traditional breeding, marker-assisted selection and genetic modification have been employed to introduce resistance genes into crop plants [12].

Traditional Breeding and Marker-Assisted selection

Traditional breeding involves the crossing of plants to produce offspring with desirable traits, including disease resistance. Marker assisted selection enhances this process by using molecular markers linked to resistance genes allowing for more precise and efficient breeding.

Genetic Modification and CRISPR Technology

Genetic modification including the use of CRISPR/Cas9 technology, allows for the direct manipulation of an organism's DNA to introduce or enhance resistance traits. This technology has been used to develop crops that are resistant to specific pathogens, reducing the need for chemical treatments [13].

Cultural Practices and Traditional Methods

Cultural Practices remain an integral part of IDM, leveraging traditional knowledge to manage plant diseases. These practices include crop rotation, intercropping sanitation and the use of disease-free planting material.

Crop Rotation and Intercropping

Crop rotation disrupts the life cycle of pathogens by alternating crops that are susceptible to different diseases. Intercropping or planting different crops in proximity can reduce disease spread by causing a less favourable environment for pathogens.

Tools of Integrated Disease Management

IDM is a holistic approach to controlling plant diseases, combing multiple strategies for sustainable, long-term disease control while minimizing economic, environmental and health impacts. Key tools in IDM include cultural practices such as crop rotation, sanitation and soil management which disrupt pathogen life cycles, remove infection sources and improve soil health. Utilizing resistant varieties through breeding or genetic modification provides plants with specific disease resistance. Biological control involves introducing beneficial microorganisms and natural predators to supress pathogens. Chemical control, although used sparingly involves the targeted application of fungicides and bactericides. Physical and mechanical methods, like barriers, traps and heat treatment, help prevent pathogen spread. Quarantine and regulatory measures enforce plant movement's restrictions to contain outbreaks. Integrated Pest Management (IPM)

emphasizes regular crop monitoring using established thresholds to glide intervention. Nutrient management through balanced fertilization and soil testing strengthens plant defences. Education and training programs for farmers supported by extension services, ensure the effective implementation of IDM practices. Combing these tools and tailoring them to local conditions enables IDM to offer effective, sustainable and environmentally friendly disease management solutions (**Figure 1**) [14].

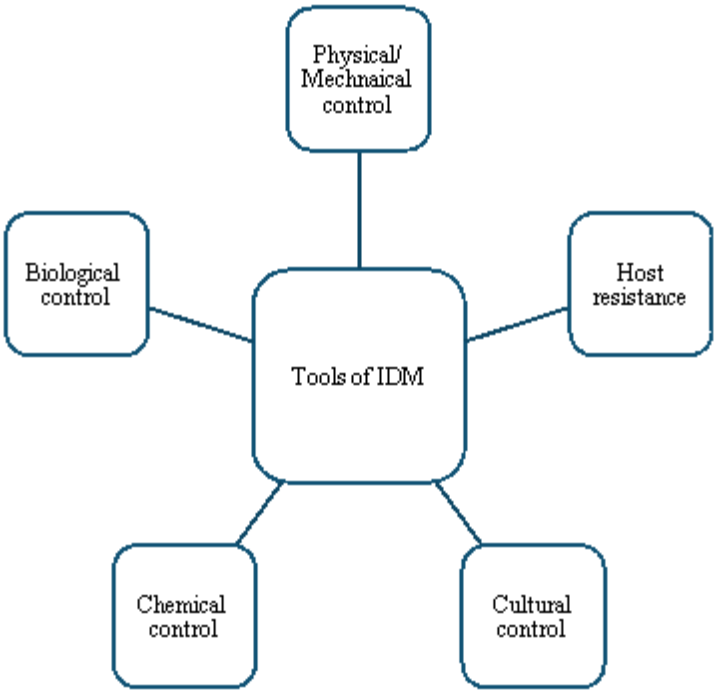

Figure1: Tools of Integrated disease management

Technological advancements in IDM

Precision Agriculture

Precision agriculture represents a major advancement in IDM, utilizing technology to optimize field-level management with regard to crop farming. This approach incorporates remote sensing, big data analytics and artificial intelligence to enhance disease monitoring and control [15].

Remote Sensing

Remote Sensing technologies such as satellite imagery and drones, provide valuable data on crop health and disease presence. These tools enable the early detection of disease symptoms, allowing for timely intervention [16].

Big Data Analytics

Big Data Analytics involves the collection and analysis of large datasets to identify patterns and trends in disease occurrence. This information can be used to develop predictive models and decision support systems for disease management [17].

Artificial Intelligence

Artificial intelligence can enhance IDM by processing complex datasets and generating insights that inform disease management decisions. AI-powered tools can analyze environmental conditions, crop health data and historical disease outbreaks to predict future disease risks and recommend appropriate intervention [18].

Role of CRISPR and Genetic modification

CRISPR technology and other genetic modification techniques continue to play a crucial role in IDM by enabling precise and targeted enhancements in crop disease resistance. These technologies offer the potential to develop crops that are not only resistant to current diseases but also adaptable to emerging threats.

Current Trends and Innovations

Sustainability and Climate Change Adaptation

As the global climate continues to change, new disease challenges emerge, necessitating resilient agricultural practices. IDM strategies increasingly emphasize sustainability and climate change adaptation, promoting practices that reduce the carbon footprint and enhance ecosystem resilience [19].

Microbial Consortia for Disease Control

Recent research has focused on the use of microbial consortia, which are communities of beneficial microorganisms that work synergistically to suppress pathogens. These consortia can enhance plant health and disease resistance through multiple mechanisms including nutrient competition and the production of antimicrobial compounds [20].

CONCLUSION

IDM represents a critical evolution in plant disease control, balancing the need for high agricultural productivity with environmental sustainability. By combining traditional knowledge with modern scientific advancements, IDM offers a robust framework for managing plant diseases in a dynamic and changing world. The continued development and implementation of IDM will be essential in addressing the dual challenges of ensuring food security and preserving the health of our ecosystems. The principles and practices of

IDM will remain central to the pursuit of sustainable agriculture, fostering resilience and adaptability in the face of emerging disease threats and global environmental changes.

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CHAPTER 2

PRINCIPLES AND COMPONENTS OF IDM

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Abstract
Integrated Disease Management (IDM) is a holistic and sustainable approach to plant disease control that combines multiple strategies to minimize crop losses while preserving environmental and economic balance. IDM emphasizes the integration of cultural, biological, mechanical, and chemical methods based on disease forecasting, economic thresholds, and ecological compatibility. The key principles of IDM include the use of resistant varieties, timely and accurate diagnosis, monitoring and surveillance, and the judicious application of pesticides when necessary. This strategy aims to reduce dependence on chemical control, delay resistance development, and promote the use of environmentally friendly practices. The components of IDM work synergistically to maintain crop health, enhance productivity, and ensure long-term agricultural sustainability. Adoption of IDM requires farmer education, interdisciplinary collaboration, and supportive policy frameworks.

Keywords: Integrated Disease Management, Disease resistance, Cultural practices, Biological control

Introduction to Integrated Disease Management (IDM)

Integrated Disease Management (IDM) is a holistic and environmentally sound approach to managing plant diseases by combining multiple strategies and practices. Rather than relying solely on chemical control, IDM integrates cultural, biological, physical, mechanical, genetic, and chemical methods to prevent and control diseases in a sustainable manner. The primary goal of IDM is to minimize crop losses while preserving environmental health, economic viability, and social responsibility. IDM emphasizes early detection, preventive measures, and rational use of fungicides or pesticides only when necessary. It encourages practices such as crop rotation, use of resistant varieties, timely sowing, proper irrigation, sanitation, and the conservation of natural enemies of pathogens.

By combining knowledge of disease biology, ecology, and epidemiology with modern agricultural technologies, IDM offers a proactive and adaptive framework for disease management. It aligns well with the principles of sustainable agriculture and supports the long-term productivity and resilience of farming systems.

2. Objectives of IDM

i. To Minimize Crop Losses Due to Diseases

IDM aims to reduce the severity and incidence of plant diseases to maintain or improve crop yield and quality.

ii. To Promote Sustainable Disease Management Practices

IDM encourages eco-friendly and sustainable methods that reduce dependence on chemical pesticides and minimize environmental impact.

iii. To Delay the Development of Resistance

By using a combination of management strategies, IDM helps prevent or low down the development of resistance in pathogens to chemical fungicides or pesticides.

iv. To Ensure Economic Viability for Farmers

IDM supports cost-effective disease management by optimizing input use and reducing unnecessary chemical applications.

v. To Conserve and Protect Natural Enemies

IDM aims to preserve beneficial microorganisms and natural antagonists that help suppress plant pathogens.

vi. To Improve Soil and Plant Health

Through practices such as crop rotation, use of organic amendments, and proper sanitation, IDM contributes to overall soil fertility and plant vigor.

vii. To Educate and Empower Farmers

One of the key objectives is to raise awareness and build capacity among farmers regarding disease identification, monitoring, and management using integrated approaches.

viii. To Support Environmental and Human Health

Reducing chemical pesticide use in IDM minimizes risks to human health, non-target organisms, and ecosystems.

3. Principles of Integrated Disease Management

IDM is guided by several core principles:

3.1. **Prevention and Exclusion in Integrated Disease Management (IDM)**

Prevention and exclusion are fundamental components of IDM aimed at stopping the introduction and establishment of plant pathogens. These proactive measures help maintain crop health and reduce reliance on curative control methods.

Use of certified, disease-free seeds and planting materials

Using clean and certified seeds ensures that the crop is free from seed-borne pathogens, which can serve as a primary source of infection. This practice significantly reduces the initial disease load in the field.

Avoidance of disease introduction by strict quarantine regulations

Quarantine laws and phytosanitary measures are essential for preventing the movement of infected plant materials across regions or countries. This helps in keeping new or invasive pathogens out of disease-free areas.

Timely sowing and harvesting to escape peak infection periods

Adjusting planting and harvesting dates allows crops to avoid periods when environmental conditions favor disease outbreaks. For instance, sowing before or after the peak spore release of a fungal pathogen can reduce infection risk.

3.2 Monitoring and Surveillance in Integrated Disease Management (IDM)

Monitoring and surveillance are essential components of IDM that enable early detection of diseases and timely interventions. These practices help in assessing the severity and spread of diseases, which is critical for making informed management decisions.

Regular scouting and disease diagnosis in the field

Systematic field scouting involves visually inspecting crops at regular intervals to identify early signs of disease. Trained personnel or farmers check for symptoms such as spots, wilting, discoloration, or unusual growth. Accurate field diagnosis helps in taking immediate action to prevent further spread and reduces the reliance on blanket pesticide use.

Use of weather-based forecasting models to predict disease outbreaks

Many plant diseases are closely linked to specific climatic conditions such as humidity, temperature, and rainfall. Weather-based forecasting models use this data to predict when and where a disease outbreak is likely to occur. These models alert farmers and extension workers in advance, enabling them

to apply preventive measures such as fungicide sprays or cultural controls at the right time.

3.3. Threshold Levels in Integrated Disease Management (IDM)

The concept of threshold levels is central to effective and economical disease management. It ensures that control measures are applied only when necessary, reducing unnecessary pesticide use and associated costs.

Application of control measures only when the disease incidence crosses the Economic Threshold Level (ETL)

The Economic Threshold Level (ETL) is the point at which the cost of potential crop loss due to disease becomes equal to or greater than the cost of control measures. In IDM, actions such as pesticide application or biological control are initiated only when the disease incidence or severity reaches this threshold.

This approach helps to:

- Prevent indiscriminate use of chemicals.
- Avoid disruption of natural biological control agents.
- Delay the development of pathogen resistance.
- Promote environmental and economic sustainability.

By monitoring disease intensity and using ETL as a decision-making tool, farmers can achieve efficient and judicious disease control.

3.5. Host Resistance

Host resistance is one of the most effective, economical, and environmentally safe strategies in disease management. It involves the use of crop varieties that are either resistant or tolerant to specific pathogens.

Deployment of disease-resistant or tolerant crop varieties

By growing varieties that are genetically resistant or tolerant to certain diseases, the risk of infection and spread is significantly reduced. These varieties can either prevent pathogen establishment (resistance) or endure infection without severe yield loss (tolerance).

Key benefits include:

- Reduced reliance on chemical pesticides.
- Long-lasting and passive protection throughout the crop cycle.
- Compatibility with other IDM practices.

Examples:

- Wheat varieties resistant to rusts (e.g., PBW 343 with leaf rust resistance).
- Rice varieties resistant to blast and bacterial blight.

- Tomato hybrids tolerant to wilt and early blight.

3.6. Environmental Considerations

Understanding the interaction between pathogens, host plants, and the environment is critical for effective disease management. Environmental conditions greatly influence the development, survival, and spread of plant pathogens.

Understanding pathogen ecology, lifecycle, and environmental factors to disrupt disease progression

Knowledge of the pathogen's lifecycle—such as modes of survival (e.g., in soil or crop debris), reproduction, and dispersal—helps in identifying vulnerable stages where interventions are most effective. Environmental factors like temperature, humidity, rainfall, and soil moisture directly impact the onset and severity of diseases.

By monitoring these factors, farmers and extension workers can:

- Time preventive measures (e.g., fungicide sprays before favorable conditions occur).
- Modify cultural practices such as irrigation, spacing, and crop rotation to create less favorable environments for pathogens.
- Break the disease cycle by removing alternate hosts, deep plowing to bury inoculum, or practicing summer fallowing.

4. Components of Integrated Disease Management

4.1. Cultural Practices

Cultural practices are foundational to IDM, focusing on modifying farming operations to make the environment less conducive for pathogen survival, development, and spread. These methods are cost-effective, environmentally friendly, and easy to adopt by farmers.

Crop rotation

Growing non-host crops in succession helps to break the life cycle of soil-borne pathogens by depriving them of their preferred host, thereby reducing the inoculum load in the soil.

Field sanitation

The removal of infected crop residues, weeds, and alternate hosts helps eliminate potential sources of primary and secondary infections. This reduces overwintering of pathogens and vector populations.

Proper plant spacing

Maintaining adequate spacing between plants improves air circulation and

reduces humidity, which is unfavorable for the development of many foliar diseases like blights and mildews.

Altered sowing and harvesting dates

Adjusting planting and harvesting times can help avoid periods of high pathogen activity or peak environmental conditions favorable for disease outbreaks, such as high humidity or rainfall.

4.2. Host Plant Resistance

Host plant resistance is one of the most reliable and environmentally sustainable strategies in IDM. It involves the use of crop varieties that are genetically capable of resisting or tolerating specific diseases.

Use of genetically resistant or tolerant cultivars reduces the need for external inputs

Resistant or tolerant varieties can suppress pathogen development or limit disease impact without the need for frequent chemical interventions. This reduces production costs, lowers chemical residue in food and soil, and supports ecological balance.

Durable resistance management to avoid breakdown of resistance

Pathogens can evolve and overcome resistance if the same variety or resistance gene is used repeatedly. To ensure durable resistance, it's important to:

- Rotate or pyramid different resistance genes.
- Use integrated approaches combining resistance with cultural, biological, or chemical methods.
- Monitor pathogen populations for changes in virulence.

4.3. Biological Control

Biological control is a key component of IDM that utilizes living organisms to suppress plant pathogens. It is an eco-friendly and sustainable alternative to chemical pesticides.

Use of natural enemies like *Trichoderma* spp., *Pseudomonas fluorescens*, *Bacillus subtilis*, and mycorrhizae to suppress disease-causing organisms

These beneficial microbes act through various mechanisms such as:

- **Antibiosis** – Production of antimicrobial compounds that inhibit pathogens (e.g., *Trichoderma*, *Pseudomonas*).
- **Competition** – Outcompeting pathogens for nutrients and space in the rhizosphere.
- **Parasitism** – Directly attacking and degrading fungal pathogens (e.g., *Trichoderma* spp. parasitizing *Rhizoctonia* and *Sclerotium*).

- **Induced Systemic Resistance (ISR)** – Stimulating the plant's own defense mechanisms (e.g., *Pseudomonas fluorescens* and *Bacillus subtilis*).
- **Symbiotic Association** – Mycorrhizal fungi improve nutrient uptake and enhance plant resistance to root pathogens.

4.4. Mechanical and Physical Methods

- Removal and destruction of infected plant parts.
- Soil solarization using polythene sheets.
- Hot water or steam treatment of seeds and planting materials.

4.5. Chemical Control

Mechanical and physical methods play a preventive role in IDM by directly eliminating or reducing the presence of pathogens and infected plant materials. These techniques are simple, cost-effective, and suitable for both small and large-scale farming systems.

Removal and destruction of infected plant parts

Regular pruning or uprooting of diseased leaves, stems, or entire plants helps to prevent the spread of infection within the field. Proper disposal by burning or deep burial is crucial to avoid re-infestation.

Soil solarization using polythene sheets

This method involves covering moist soil with transparent polythene sheets during peak summer months for 4–6 weeks. The trapped solar radiation raises the soil temperature, effectively killing many soil-borne pathogens, weed seeds, and nematodes.

Hot water or steam treatment of seeds and planting materials

Hot water treatment (usually at 50–55°C for specific durations) and steam treatments are used to eliminate seed-borne pathogens without damaging the seed viability. This is commonly applied to crops like rice, sugarcane, and vegetable seedlings.

4.6. Legislative and Quarantine Measures

Legislative and quarantine measures are formal, government-enforced actions aimed at preventing the introduction and spread of exotic or economically damaging plant diseases across regions and national borders.

Government regulations to prevent the spread of exotic and harmful pathogens

Laws such as the Destructive Insects and Pests Act (DIP Act, 1914) in India empower authorities to restrict or regulate the import and movement of plant materials that may carry dangerous pathogens. These measures help prevent

the entry of quarantine pests and diseases like citrus greening, Karnal bunt of wheat, or bacterial wilt in solanaceous crops.

Inspection and certification systems to ensure clean planting material

Certified seed and planting material programs involve regular inspection, testing, and certification to guarantee that only disease-free materials are used by farmers. This reduces the risk of introducing seed-borne or propagule-borne pathogens into new areas.

4.7. Disease Forecasting and Early Warning Systems

Forecasting and early warning systems are proactive tools in IDM that help in anticipating disease outbreaks and enabling timely, targeted interventions—thereby minimizing crop losses and unnecessary pesticide use.

Use of weather data and predictive models to forecast disease outbreaks and plan interventions

Many plant diseases are triggered or aggravated by specific environmental conditions such as temperature, humidity, rainfall, and wind. Disease forecasting systems collect and analyze this weather data through:

- Epidemiological models that predict pathogen development stages.
- Decision support tools that advise on when and where to apply control measures.
- GIS and remote sensing data integration for spatial disease risk mapping.

Examples include:

- **FAST** (Forecasting Alternaria solani in Tomato)
- **BLIGHTCAST** for late blight in potato
- **DOWNCAST** for downy mildew in grapevines

These systems help in:

- Reducing unnecessary chemical use by targeting sprays only when needed.
- Saving costs and protecting the environment.
- Improving preparedness among farmers and extension workers.

5. Advantages of IDM

IDM offers a holistic approach to plant disease control by combining cultural, biological, mechanical, and chemical strategies in a balanced manner. Its adoption results in numerous agronomic, environmental, and economic benefits.

Reduces production cost and enhances yield quality

By relying more on preventive and non-chemical methods, IDM helps farmers reduce input costs. Healthier crops with fewer disease-related losses also result in improved yield quantity and quality**.**

Minimizes chemical residues in food and the environment

Judicious and need-based application of pesticides in IDM reduces the accumulation of harmful chemical residues in produce, soil, and water, ensuring safer food and a cleaner environment.

Preserves beneficial organisms and biodiversity

IDM promotes the use of selective and biological control methods that are less disruptive to natural enemies**,** pollinators, and other beneficial soil and crop-inhabiting organisms, thereby conserving ecosystem balance**.**

Slows down the development of pesticide resistance in pathogens

Frequent and indiscriminate pesticide use accelerates resistance in pathogens. IDM reduces this pressure through rotational and integrated use of various control methods**,** helping maintain pesticide efficacy over time**.**

Promotes sustainable and profitable farming practices

By enhancing soil and plant health, reducing input costs, and ensuring long-term productivity, IDM supports sustainable, eco-friendly, and economically viable agriculture**.**

6. Challenges in Implementing IDM

While IDM offers significant benefits, its widespread implementation faces several practical and systemic challenges that must be addressed for it to be fully effective.

Lack of awareness and technical knowledge among farmers

Many farmers, especially in remote or resource-poor regions, are unaware of IDM principles **or** lack access to training on its practical implementation. This results in a continued reliance on chemical control methods.

Limited availability of resistant varieties and biocontrol agents

In some areas, disease-resistant crop varieties or effective biocontrol products may be unavailable or not adapted to local conditions, limiting farmers' ability to adopt key IDM components.

Inadequate forecasting systems and diagnostic infrastructure

Reliable disease surveillance, diagnostic labs, and forecasting models are not uniformly available or accessible, reducing the effectiveness of timely and location-specific interventions.

Difficulty in managing complex disease complexes under field conditions
Crops are often affected by multiple pathogens simultaneously, influenced by changing climate and cropping practices. This makes it difficult to design one-size-fits-all IDM strategies, especially under diverse and dynamic field conditions.

7. Case Example: IDM in Rice Blast Disease

Pathogen: *Magnaporthe oryzae*

IDM Strategy:

- **Resistant varieties**: Use of blast-resistant rice hybrids.
- **Cultural control**: Water management, balanced fertilization, and removal of infected debris.
- **Biocontrol**: Application of *Trichoderma harzianum*.
- **Chemical control**: Use of systemic fungicides like tricyclazole during early infection.

8. Conclusion

Integrated Disease Management (IDM) represents a sustainable and holistic approach to managing plant diseases by combining cultural, biological, mechanical, physical, legislative, and chemical strategies in a coordinated manner. It emphasizes prevention, timely monitoring, and the use of eco-friendly techniques to reduce crop loss and minimize environmental impact. Throughout this overview, we have seen how IDM:

- Enhances yield quality and profitability by reducing disease pressure with minimal reliance on chemicals.
- Promotes environmental and food safety through reduced pesticide residues.
- Supports biodiversity and natural enemies, preserving the ecological balance in agroecosystems.
- Plays a vital role in sustainable agriculture by slowing resistance development and promoting long-term productivity.

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CHAPTER 3

INTEGRATED DISEASE MANAGEMENT FOR SUSTAINABLE AGRICULTURE

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Abstract

Integrated Disease Management (IDM) is a holistic approach to control and prevent diseases across various domains, including agriculture and human health. This strategy involves the use of a combination of methods, such as biosecurity measures, vaccination programs, and cultural practices, to effectively manage diseases and reduce their impact. The historical overview highlights traditional, chemical, and integrated approaches to manage plant diseases. IDM seeks to optimize resource use and enhance disease control outcomes by integrating diverse techniques and tools. By addressing the interactions among diseases, hosts, and environments, IDM fosters a comprehensive and collaborative approach to disease management, leading to healthier and more resilient systems. Additionally, the chapter addresses institutional, sociological, economic, and political constraints related to integrated disease management.

Keywords: Integrated Disease Management, Biosecurity, Human Health, Agriculture, Vaccination.

1. Introduction

Integrated Disease Management (IDM) is a comprehensive and sustainable method for effectively controlling plant diseases in agriculture while reducing negative environmental impacts and ensuring long-term productivity. This science-based strategy combines various disease management practices to achieve optimal outcomes. IDM seeks to balance economic viability, environmental preservation, and social benefits. It focuses on prevention, early detection, and the use of diverse tools to keep plant populations healthy and maximize yields. This approach is essential in modern agriculture, where

minimizing chemical use and fostering sustainable practices are critical. IDM can be described as a disease management system that, considering the associated environment and the dynamics of microorganism populations, employs all appropriate techniques in a compatible manner to keep disease levels below economically damaging thresholds [1]. Integrated Disease Management for sustainable agriculture offers a holistic strategy for managing plant diseases that reduces impacts on the environment, economy, and society while enhancing long-term agricultural productivity [2]. Here's an in-depth exploration of how IDM can be implemented to foster sustainable farming practices:

1.1. Essential Elements of Integrated Disease Management:

1.1.1. Monitoring and Early Detection:

- **Regular Scouting:** Frequent field inspections facilitate the early identification of disease outbreaks.
- **Diagnostic Tools:** Employ advanced diagnostic technologies, such as molecular assays and remote sensing, for accurate disease identification [3].

1.1.2. Cultural Practices:

- **Crop Rotation:** Alternating crops to disrupt pathogen life cycles.
- **Resistant Varieties:** Planting varieties that are resistant to specific diseases.
- **Sanitation:** Properly disposing of infected plant material and cleaning equipment to prevent disease spread.
- **Field Management:** Spacing plants appropriately to enhance air circulation and reduce humidity, which helps inhibit disease development.

1.1.3. Biological Control:

- **Beneficial Organisms:** Introducing or fostering natural predators, parasites, or antagonists of plant pathogens.
- **Microbial Inoculants:** Using beneficial microorganisms to improve plant health and suppress pathogens [4].

1.1.4. Chemical Control:

- **Judicious Use of Pesticides:** Applying fungicides, bactericides, or other chemicals in a targeted, minimal manner, ideally as a last resort.
- **Resistance Management:** Avoiding overuse of a single chemical to prevent the emergence of resistant pathogen strains.

1.1.5. Genetic Control:

- **Breeding for Resistance:** Developing and using crops with inherent genetic resistance to specific diseases.
- **Genomic Tools:** Leveraging genetic information to better understand resistance mechanisms and enhance breeding programs.

1.1.6. Integrated Pest Management (IPM):

- **Combining Tactics:** Employing a combination of biological, cultural, mechanical, and chemical methods to manage pest populations and reduce disease risk.
- **Thresholds and Action Levels:** Setting thresholds to determine when control measures should be implemented based on pest and disease levels [5].

1.1.7. Education and Training:

- **Farmer Training:** Providing education on disease identification, management practices, and emerging technologies.
- **Extension Services:** Offering continuous support and resources through agricultural extension services.

1.1.8. Environmental Considerations:

- **Sustainable Practices:** Adopting practices that minimize environmental impact, such as reducing chemical use and enhancing biodiversity.
- **Climate Adaptation:** Adjusting practices to accommodate changing climate conditions that may affect disease prevalence and severity [6]**.**

1.2. Benefits of Integrated Disease Management:

- **Reduced Environmental Impact:** IDM minimizes the need for excessive pesticide use by employing a range of methods, thereby decreasing ecological harm.
- **Increased Resilience:** Strengthens the ability of crops and farming systems to withstand diseases, resulting in more consistent yields and stable income.
- **Economic Efficiency:** Lowers disease management costs by integrating various strategies and decreasing dependence on costly chemical treatments.

- **Sustainable Production:** Supports long-term agricultural sustainability through the preservation of soil health, conservation of biodiversity, and maintenance of ecosystem health [7].

1.3. Challenges and Considerations:

- **Complexity:** Implementing IDM can be intricate, necessitating a thorough understanding of the interactions among crops, pathogens, and environmental factors.
- **Resource Intensive:** Successful IDM often demands investment in training, technology, and research.
- **Adaptability:** Strategies must be flexible to accommodate local conditions and evolving disease threats [8].

1.4. Importance of Integrated Disease Management:

- **Sustainable Agriculture:** IDM fosters sustainable agricultural practices by reducing dependency on chemical pesticides and promoting environmentally friendly disease management methods. This approach helps conserve natural resources, maintain biodiversity, and support the overall health of agroecosystems.
- **Resilience to Disease Outbreaks:** By integrating various disease management strategies, IDM builds a more resilient agricultural system capable of better handling disease outbreaks. Crop diversity and cultural practices help lessen the impact of specific diseases on entire crops.
- **Reduced Chemical Use:** IDM significantly cuts down on the need for chemical pesticides by combining cultural practices, biological controls, and disease-resistant plant varieties. This reduction in chemical use decreases residues in crops, soil, and water, benefiting human health and ecosystems.
- **Economic Viability:** IDM seeks to optimize disease management methods, which lowers overall disease control costs while maintaining or improving crop yields and quality, thereby enhancing the economic viability of farming operations.
- **Reduced Environmental Impact:** By minimizing chemical pesticide use, IDM helps reduce environmental pollution and mitigates harmful effects on beneficial organisms such as pollinators and natural pest predators.
- **Public Health and Safety:** IDM promotes the safe and judicious use of pesticides, reducing risks to farm workers, consumers, and the environment.

- **Innovation and Research:** The application of IDM encourages ongoing research and innovation in disease management, leading to the development of new technologies, biological control agents, and disease-resistant crop varieties.
- **Adaptation to Climate Change:** IDM supports a diverse and adaptable agricultural system, which is crucial for addressing the challenges posed by climate change and emerging diseases.
- **Preserving Beneficial Organisms:** The biological control methods used in IDM help preserve natural enemies of pests and diseases, maintaining ecosystem balance. This preservation can reduce secondary pest outbreaks and contribute to a more resilient agroecosystem.
- **Integrated Approach:** IDM acknowledges the complexity of disease interactions and employs a comprehensive strategy. Instead of relying on a single method, IDM combines various tools to tailor disease management strategies to specific crops, regions, and conditions [7].

2. History of Integrated Disease Management:

The evolution of Integrated Disease Management (IDM) reflects the progression of agricultural practices and our understanding of plant diseases. While the integration of various disease management strategies has ancient roots, IDM as a formalized, systematic approach began to develop in the 20th century [9].

- **Early Agricultural Practices:** Historically, farmers used various techniques to manage plant diseases, often without realizing they were employing elements of what is now known as IDM. Practices such as crop rotation, intercropping, and selecting disease-resistant plants were commonly used to mitigate disease impacts.
- **Emergence of Modern Plant Pathology:** The late 19th and early 20th centuries saw the establishment of modern plant pathology as a scientific discipline. Researchers began systematically studying the causes, mechanisms, and control of plant diseases.
- **Shift from Chemical Control to Integrated Approaches:** In the mid-20th century, the extensive use of synthetic pesticides for disease control became prevalent. However, the limitations of relying solely on chemical methods, such as pesticide resistance and environmental issues, soon became apparent.
- **1960s-1970s: Early Formulation of IDM:** During this period, the drawbacks of chemical control led to the recognition of the need for a more

comprehensive approach. The concept of "Integrated Pest Management" (IPM) emerged, combining biological, cultural, and chemical strategies for pest and disease control. This idea evolved from the "integrated control" concept proposed by entomologists at the University of California.

- **1980s-1990s: Expansion of IDM:** IDM began to be identified as a specific component of IPM, with a focus on plant disease management. Researchers and agricultural extension services promoted the integration of various approaches, such as resistant plant varieties, cultural practices, and biological controls, to manage plant diseases more effectively.
- **21st Century: Advancements and Emphasis on Sustainability:** The 21st century brought increased attention to sustainable agriculture and the role of IDM in achieving it. Global concerns about environmental impact, food safety, and chemical use led to a stronger emphasis on integrated disease management approaches.
- **Current State and Future Directions:** Today, IDM is a standard practice in modern agriculture, emphasizing the combination of multiple strategies to manage plant diseases effectively while minimizing environmental and health impacts. Ongoing research and technological advancements continue to refine and enhance IDM practices.

  In summary, Integrated Disease Management has evolved from traditional practices into a scientifically grounded, holistic approach that addresses the complexity of plant-disease interactions. It focuses on integrating diverse strategies to achieve sustainable and environmentally friendly agricultural outcomes.

3. Concepts of Integrated Disease Management (IDM):

Integrated Disease Management (IDM) is a comprehensive and sustainable strategy for controlling plant diseases in agriculture, aiming to minimize environmental impacts while ensuring long-term productivity. It involves the combination of various disease management techniques to achieve optimal outcomes [10]. The key concepts of IDM include:

- **Holistic Approach:** IDM adopts a holistic perspective, considering the entire agroecosystem—including crops, soil, climate, pests, beneficial organisms, and cultural practices. It targets the root causes of diseases rather than merely addressing symptoms.
- **Disease Management Tactics:** IDM integrates a range of disease management tactics such as cultural practices, resistant crop varieties,

biological control, and, when necessary, chemical treatments and monitoring systems to create a synergistic effect in disease control.

- **Sustainable Practices:** IDM promotes sustainable agricultural practices by reducing reliance on chemical pesticides and encouraging environmentally friendly disease management methods.
- **Crop Diversification:** A key component of IDM, crop diversification reduces the risk of widespread disease outbreaks and strengthens the resilience of the agricultural system.
- **Economic Viability:** IDM focuses on optimizing disease management strategies to lower costs while maintaining or improving crop yields and profitability for farmers.
- **Adaptability:** IDM advocates for adaptable disease management approaches, recognizing that different crops, regions, and environmental conditions may require customized solutions.
- **Prevention and Early Detection:** IDM emphasizes the importance of preventing disease outbreaks through practices like crop rotation, sanitation, and the use of disease-resistant varieties. Regular monitoring facilitates early disease detection and timely intervention.
- **Diverse Strategies:** IDM employs a mix of strategies, including cultural methods, biological controls, resistant plant varieties, chemical treatments, and monitoring techniques, to effectively manage and control diseases.
- **Site-Specific Management:** IDM tailors disease management plans to local conditions such as climate, soil, and cropping systems, addressing the specific needs of each location.
- **Stakeholder Collaboration:** Effective IDM often involves collaboration among researchers, extension services, farmers, and other stakeholders, fostering a cooperative approach to disease management.

4. Principles of Integrated Disease Management:

- **Prevention First:** The core principle of IDM is prevention. Prioritize preventive measures such as employing disease-resistant crop varieties, implementing precise crop rotation, and maintaining excellent sanitation to minimize disease occurrence.
- **Diversity and Resilience:** Promote crop diversification to lower the risk of disease outbreaks and enhance the overall resilience of the agroecosystem.

- **Threshold-Based Control:** Utilize monitoring systems to establish economic injury thresholds, intervening only when disease levels surpass a set economic threshold.
- **Integration of Tactics:** Combine various disease management tactics—such as cultural practices, biological controls, and chemical treatments—to achieve a synergistic effect and optimize disease control.
- **Environmentally Friendly Practices:** Favor environmentally friendly and less-toxic disease management methods to reduce harm to non-target organisms and the environment.
- **Regular Monitoring:** Consistently monitor crops for signs of disease and assess severity to detect issues early and make informed management decisions [11].

5. Strategies and Tools of Integrated Disease Management:

Integrated Disease Management (IDM) employs a range of strategies and tools to manage and control plant diseases effectively while supporting sustainable agriculture. These strategies involve various methods to tackle disease-related challenges, including:

5.1. Resistant Host Plant Varieties:

- Utilizing plant varieties that are naturally resistant to specific diseases is a key component of IDM. Breeding for resistance focuses on developing cultivars with genetic traits that offer protection against pathogens.
- Resistant varieties provide a practical, effective, and economical method for controlling plant diseases. They not only safeguard plants but also save time, money, and effort by reducing the need for other control measures and minimizing environmental pollution from chemicals.
    - For diseases like wilts and rusts, or viral diseases where chemical control is costly and impractical, resistant varieties are often the most viable solution.
    - In low-value crops, where other control methods might be too expensive, developing disease-resistant varieties can be a valuable recommendation for farmers.
    - Disease resistance in plants can be due to their genetic makeup and may be monogenic, oligogenic, or polygenic [12].

5.2. Cultural Practices: Cultural practices are crucial for disease prevention and include methods like crop rotation, proper spacing, optimizing planting density, adjusting irrigation practices, and maintaining good sanitation.

- **Deep Ploughing:** This exposes pathogen propagules to high temperatures and physically destroys them, acting like dry soil solarization. Summer ploughing has been effective in reducing cyst nematode populations and improving wheat yields.
- **Flooding:** Long-term summer soil flooding, with or without paddy culture, can decrease populations of soil-borne pathogens.
- **Crop Rotation and Diversification:** Alternating crops in a field disrupts pathogen life cycles and reduces disease pressure, promoting a balanced ecosystem.
- **Residue Management:** Removing and disposing of infected plant debris, plowing under crop residues, and practicing crop rotation help reduce pathogen survival and spread.
- **Other Cultural Practices:** To minimize soil-borne pathogen dispersal, equipment and stakes should be decontaminated between fields. Avoid moving soil between sites to reduce pathogen spread. Effective weed control is vital for managing viral diseases, and avoiding plant injuries can prevent pathogen entry. Removing plant material after harvest can also reduce pathogen inoculum.

5.3. Biological Control Agents: Beneficial organisms, including predators, parasitoids, and helpful microbes, are used to manage disease-causing organisms and maintain a balanced ecosystem [13].

- **Biocontrol Agents:** These are integral to IDM systems and include organisms such as *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Gliocladium spp.*
- **Biopesticides:** Derived from natural sources like fungi, bacteria, and plant extracts, biopesticides offer an alternative to synthetic chemicals and can effectively control diseases.
- **Predators and Parasites:** Some insects and mites feed on plant pathogens, such as predatory nematodes attacking harmful soil nematodes, and parasitic wasps laying eggs inside pest insects.
- **Competition:** Introducing non-pathogenic strains of a pathogen can create competition for resources, hindering the harmful strain's establishment.

- **Induced Resistance:** Beneficial microorganisms can trigger the plant's natural defense mechanisms, enhancing its resistance to diseases.

5.4. Physical Control: Physical control methods involve using barriers or techniques to prevent disease spread. Examples include row covers, mulching, and sanitation practices to limit disease transmission [14].

- **Collect and Destroy Infected Plant Parts:** Removing and disposing of disease-infected plant parts is crucial for managing plant diseases.
- **Soil Solarization:**
  - Soil solarization is an effective method for controlling soil-borne diseases caused by fungi that are otherwise difficult to manage, such as *Rhizoctonia solani*, *Fusarium* spp., and *Sclerotium*.
  - The process involves irrigating soil beds and then covering them with thin (20 µm) transparent mulch during April, May, and June.
  - This method raises soil temperatures, sometimes up to 50°C, which can be harmful to many soil pathogens.
  - Soil solarization is also used to produce disease-free nurseries in tropical and subtropical regions and provides excellent weed control.
- **Hot Water Treatment:**
  - Hot water treatment can address some seed-borne diseases by immersing infected seeds in hot water at a specific temperature and duration.
  - For example, treating cabbage seeds at 52°C for 15-20 minutes effectively controls black rot caused by Xanthomonas campestris pv. campestris.
- **Hot Air Treatment:**
  - Hot air treatment removes excess moisture from plant organs and protects them from fungal and bacterial infections.
  - Dormant plants infected with certain viruses can be treated with hot air at temperatures ranging from 35-54°C for 8 hours.
- **Refrigeration (Low Temperature Treatment):**
  - Refrigeration is commonly used to prevent postharvest diseases in perishable fruits and vegetables.
- **Solar Heat Treatment:**
  - Solar heat treatment involves soaking wheat seeds in water and exposing them to solar heat for 5-6 hours during May-June, which effectively controls loose smut of wheat.

- Postharvest diseases can be managed using methods such as irradiation, refrigeration, and controlled atmosphere storage.

5.5. Chemical Control (As a Last Resort): When other methods are inadequate, carefully chosen chemical pesticides may be used as a last resort. It is crucial to apply these chemicals thoughtfully, taking into account factors such as their specificity for the target pathogen, the timing of application, and their potential effects on non-target organisms [15].

- **Types of Chemicals:**
- **Fungicides:** Used to manage fungal diseases.
- **Bactericides:** Target bacterial infections.
- **Insecticides:** Address insect pests.
- **Nematicides:** Control nematode pests.
- **Virucides:** Aim to manage viral diseases, though options are limited**.**
- **Seed Treatment:** Treating seeds with fungicides, biological agents, or other substances before planting can offer early protection against soil-borne diseases.
- **Disease Thresholds:** Disease thresholds are used to determine when intervention is necessary to avoid economic losses. These thresholds guide farmers in making informed decisions about when to implement disease management strategies.

5.6. Quarantine & Regulatory Measures:

- Preventing the introduction and spread of exotic pathogens through quarantine and regulatory measures is a key component of Integrated Disease Management (IDM).
- Plant quarantine involves legally restricting the movement of diseased plant materials and pathogens, such as fungi, bacteria or viruses that cause plant diseases.
- Quarantine and regulatory measures are tools of exclusion, aligning with the principles of plant disease control.

6. Integrated disease management in greenhouses

Integrated disease management in greenhouses involves employing a variety of strategies to prevent and control crop diseases. This approach uses hazard analysis to pinpoint potential infection risks, allowing for the implementation of preventative or corrective measures to reduce the chance of disease spread. Throughout the cropping cycle, regular monitoring helps determine the necessity and type of intervention required.

Currently, the term Integrated Pest Management (IPM) encompasses a broad range of integrated practices for managing various pests, including diseases. IPM is a key component of effective agricultural practices aimed at producing crops profitably and sustainably.

A plant disease disrupts normal plant functioning or development. For a disease to manifest, three conditions must be met: the presence of a pathogen on or within the plant, suitable environmental conditions for the pathogen, and a plant that is susceptible to the disease [16].

6.1. Hygiene

- Effective disease management in greenhouses begins with preventing pathogens from reaching the crops. Ensure that all materials, containers, and equipment entering the greenhouse are clean. Refer to guidelines on preventing pests and diseases in greenhouses for more detailed practices.

- Install and maintain a foot bath at every greenhouse entrance. While commercial foot bath pads are available, a container with foam and a disinfectant solution is also effective. Ensure that everyone entering the greenhouse uses the foot bath each time they enter, and replace the disinfectant solution at least every two weeks, or more frequently if it becomes muddy.
- Although it may not be possible to exclude all pathogens from a greenhouse—since some diseases may first appear near vents due to airborne spores—maintaining good hygiene practices will significantly reduce disease-related losses.
- Additionally, workers who smoke should wash their hands thoroughly with medicated soap before entering the production area, as some viruses and bacteria can be transferred to plants through touch.
- If possible, empty and clean the greenhouse thoroughly between crops. Avoid using formalin due to its harmful fumes, which can damage plants and pose health risks to workers. Greenhouses that are kept clean generally experience fewer disease issues.

6.2. Control Entry

- Limit access to the greenhouse since pathogens and pests can easily be carried on clothing and shoes. Diseases often first appear near doorways. Reducing the number of people entering the greenhouse decreases the likelihood of introducing pathogens and pests.

- When visitors are present, provide disposable overalls and avoid allowing visitors who have recently been in other greenhouses. When moving between crops, always start with the youngest and healthiest plants before proceeding to older, potentially infected crops to minimize the risk of spreading pathogens.

6.3. Start with Disease-Free Plants

- Inspect seedlings upon delivery. Report any signs of disease to the supplier immediately. Remove diseased plants, place them in sealed plastic bags, and submit them for diagnostic testing. Store seedlings in a designated clean area before transplanting. Check new plants for pests or diseases before introducing them to the greenhouse, and do not plant any that appear diseased or infested.
- Opt for crop varieties resistant to pests and diseases when possible. While many cultivars offer resistance to diseases like Powdery Mildew, Downy Mildew, and viruses, no variety is immune to all diseases.

6.4. Control the Growing Environment

- Adjusting the greenhouse environment to make it less conducive to disease organisms is a highly effective control method. Proper temperature and humidity management are crucial for minimizing diseases such as Downy and Powdery Mildew, and Botrytis. Guttation, where pathogens are picked up by leaf exudates, can lead to infections, so managing humidity and preventing excess moisture is important.
- Condensation, tissue damage from pruning, and other forms of plant stress can make plants more susceptible to infections. Condensation can also dilute fungicide applications, potentially contributing to pathogen resistance.

6.5. Inspect Plants Regularly

- Regular crop monitoring facilitates early disease detection and improves control strategies. Inspect at least 5% of plants in each row and pay attention to areas with localized “microclimates,” such as shadier spots or places where moisture accumulates. Use colored plastic tape or ribbons to mark these "hot spots" for targeted treatment, reducing the need to treat the entire crop.

6.6. Waste Management

- Promptly remove and destroy crop residues after pruning and harvest. Avoid piling plant material near the greenhouse; instead, place it directly into bags or a rubbish skip bin for disposal. Ensure the bin is emptied

regularly to prevent pathogen breeding. If burying debris, do so immediately and avoid stockpiling. Check local regulations before burning crop debris, as it may be restricted.

6.7. Control Insects and Weeds

- Manage insects and weeds both inside and outside the greenhouse. Weeds can harbor diseases and pests, while insects can carry diseases. If feasible, install insect screens over greenhouse openings, though be aware that screens may reduce air flow and impact ventilation. Poor air circulation can contribute to diseases such as Botrytis, Alternaria, and Downy Mildew. A double-door entry with a foot bath helps reduce pest and disease entry.

6.8. Fungicides

Fungicides come in two types: protectants and eradicants.

- **Protectants:** These remain on the plant surface and control pathogens through direct contact. They are effective against a broad range of fungal pathogens and need to be reapplied regularly as new plant growth emerges. Ensure thorough and even coverage when using protectants.
- **Eradicants (Curatives):** These systemic fungicides are absorbed by plants and control pathogens at sites beyond where the chemical is applied. They move into new growth, requiring less frequent application than protectants. However, their specific action can lead to resistance in pathogens, as seen with Downy Mildew, Powdery Mildew, and Grey Mold.

Both types of fungicides can spread pathogen spores within the greenhouse through spray mist. If the pathogen is resistant to the fungicide, spraying can exacerbate the problem. Copper fungicides, for example, are known for such issues.

7. Conclusion

Integrated Disease Management (IDM) is a holistic and sustainable strategy designed to manage plant diseases effectively while safeguarding the environment and maintaining economic viability. Its evolution from traditional pest control methods to a more comprehensive approach highlights its significance in contemporary agriculture. IDM integrates multiple disease management tactics to control plant diseases while minimizing environmental impact.

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CHAPTER 4

PROTEOMICS AND METABOLOMICS IN PLANT DISEASE MANAGEMENT

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Abstract
Plant diseases jeopardise agricultural productivity and food safety. Traditional methods, such as chemical treatments and breeding for resistant varieties, frequently fail due to pathogen evolution and environmental concerns. Proteomics and metabolomics provide molecular insights into plant-pathogen interactions, which improve disease management strategies. Proteomics investigates protein changes in response to infection, whereas metabolomics examines metabolic shifts to reveal defence mechanisms. Advances in mass spectrometry and bioinformatics have helped us better understand these interactions. Integrating 'omics' data with traditional practices can result in long-term solutions like disease-resistant crops, early detection biomarkers, and natural product-based pesticides.

Key words: Plant Disease Management, Proteomics, Metabolomics, Disease Resistance, Bioinformatics, Sustainable Agriculture

Introduction

A major threat to food security, plant diseases is typically caused by agricultural pests and pathogens and result in crop losses. Agrochemicals are effective in controlling plant diseases. However, the widespread use of chemical pesticides and fertilisers has detrimental effects on both human health and the ecosystem, leading to ecological imbalance, pathogen resistance, and environmental pollution [1]. Because bio controls use natural control agents like beneficial microorganisms and their by-products, metabolites, they are more environmentally friendly than chemical methods [2]. Because of their potential as biological control agents (BCAs), entophytes and their bioactive metabolites have drawn a lot of attention [3,4].

The machinery of the cell, proteins are responsible for almost all aspects of organismal growth, reproduction, and reactions to stress[5-6]. Proteome

analyses can provide a more accurate picture of the cell's current state than transcriptome analyses, which are useful in predicting a cell's potential. Quantitative proteomics using mass spectrometry (MS) is crucial for comprehending changes in the quantity of proteins and the variety of their post-translational modifications (PTMs) to how pathogens react to plants [7-9].

Plant responses to stress are essential for their adaptation and survival in harsh environmental circumstances. In the fields of genomics, transcriptomics, proteomics, and metabolomics, scientists and plant breeders have used a variety of potent instruments and methodologies to obtain clear, thorough insights into these responses at the molecular level [10-12].

Plant organs, tissues, and intercellular spaces are home to endophytes, which can include endophytic bacteria, fungi, and actinomycetes, without immediately posing a threat to health [13-14]. Over the course of long-term coevolution, they have developed a mutually beneficial relationship with host plants. Endophytes help to sustain the health of plants by supplying nutrients to them, and plants in turn [15-16]. Alkaloids, polypeptides, polyketides, terpenoids, and other metabolites with a wide range of biological activities, which are produced by endophytes, are very valuable and significant in many fields, particularly in agriculture and the pharmaceutical industry [17-18]. Because these metabolites can be used as natural antioxidants, antibiotics, insecticidal agents, antitumor agents, antidiabetic products, and more, they have garnered a lot of interest and attention [19-20].

These bioactive metabolites primarily aid host plants in resisting biotic and abiotic stresses, either directly or indirectly, in order to protect plant health. For instance, endophytic bacteria secrete hydrolases that can break down pathogen cell walls, some of the antimicrobial compounds produced by endophytes are well known for their potent ability to inhibit pathogens, and phytohormones released by endophytes are essential for plant development and stress response [21-22]. Plant growth-promoting microbes (PGPM) are a class of microorganisms that are well-known for their ability to elicit prime systemic resistance in plants. They achieve this by generating metabolites, which include volatile organic compounds and antimicrobials [23]. It's important to note that research on the use of endophyte metabolites to induce plant resistance is still in its infancy and needs to be investigated and improved upon in upcoming studies.

The molecular study of plant stress responses has been transformed by the use of tools and techniques from the fields of genomics, transcriptomics, proteomics, and metabolomics. In light of shifting environmental conditions, this

interdisciplinary approach offers prospects for the development of resilient crop varieties and sustainable agricultural practices by offering a comprehensive understanding of the intricate molecular networks involved in stress adaptation.

2 Literature survey

2.1 Introduction

Two cutting-edge omics technologies that have greatly improved our comprehension of plant responses to diseases are proteomics and metabolomics. These methods offer thorough insights into the metabolite and protein profiles of plants, respectively, and aid in the deciphering of intricate biochemical networks related to disease management and plant-pathogen interactions [24].

2.2 Proteomics in the Management of Plant Diseases

2.2.1 Plant Proteomics Overview

Large-scale protein research, focussing on their structures and functions, is known as proteomics. Proteomics aids in the identification of proteins that are expressed differently in plants in response to pathogen infection, the clarification of signalling pathways, and the comprehension of defence mechanisms in plants[25].

2.2.2 Pathogen Response:

Pathogenesis-related (PR) proteins, including PR1, PR2, and PR5, have been found through proteomic studies to be upregulated during pathogen attacks. According to Jwa et al. (2013), these proteins are essential for both local defence mechanisms and systemic acquired resistance (SAR).

2.2.3 Protein Modifications: The regulation of plant immune responses depends heavily on post-translational modifications (PTMs), such as phosphorylation and ubiquitination. For example, research has demonstrated that signalling cascades initiated by pathogen recognition involve protein phosphorylation (Meng et al., 2013).

2.2.4 Defence Pathways: According to proteomics, plants use a wide range of defence mechanisms, such as the generation of antimicrobial peptides, the activation of mitogen-activated protein kinases (MAPKs), and the build-up of reactive oxygen species (ROS) (Kumar et al., 2014).

2.3. Utilisations in the Management of Diseases

2.3.1 Biomarker Discovery: The identification of putative biomarkers for early disease detection has been made possible by proteomic approaches. For instance, disease resistance or susceptibility can be predicted based on the expression levels of particular proteins (Aung et al., 2019).

2.3.2 Resistance Breeding: By identifying proteins linked to resistance traits, proteomic data helps select resistant plant varieties and promotes the growth of more resilient crops (Liu et al., 2018).

2.3.3 Using Metabolomics to Manage Plant Diseases

2.3.1. Plant Metabolomics Overview:

The thorough examination of metabolites—the tiny molecules involved in metabolic pathways—is known as metabolomics. This method aids in the comprehension of the metabolic alterations brought about by pathogen infection and plant reactions [26].

2.3.1.1 Metabolic Changes: Research on metabolomics has revealed a number of metabolites, including flavonoids and phenolics, as well as salicylic acid (SA), jasmonic acid (JA), and other secondary metabolites, that build up in response to pathogen infection (Mazzoni et al., 2015).

2.3.1.2 Metabolic Pathways: Under conditions of illness stress, important metabolic pathways are altered, including the phenylpropanoid pathway. It's common to see increased production of defence compounds like phytoalexins (Wang et al., 2019).

2.3.1.3 Stress Metabolites: Certain metabolites, such as those necessary for the production of antioxidants, help to lessen the oxidative stress brought on by pathogen invasions (Kollist et al., 2019).

2.3.2. Applications for Disease Management

2.3.2.1 Disease Diagnostics: Metabolomics enables the identification of metabolic fingerprints associated with specific diseases, allowing for the development of diagnostic tools for early disease detection (Smeekens et al. 2014).

2.3.2.2 Stress Management: Metabolomics helps to develop plant stress management strategies by understanding metabolic responses to pathogens, such as the use of biostimulants or growth regulators (Kim et al., 2021).

2.4. Integrating Proteomics and Metabolomics

Combining proteomics and metabolomics yields a comprehensive picture of plant responses to pathogens. Integrated omics approaches enable a more comprehensive understanding of the interactions between proteins and metabolites during disease, providing insights into regulatory networks and potential intervention targets (Tzin et al. 2017).

2.5. Future Directions.

2.5.1 High-Throughput Technologies: Advances in high-throughput proteomics and metabolomics techniques will improve our ability to analyse

complex plant-pathogen interactions[27].

2.5.2 Systematic Biology Approaches: Integrating proteomics and metabolomics data with genomic and transcriptomic data can provide a more comprehensive picture of plant immune responses and aid in the development of new disease management strategies.

Field Applications: It is still difficult to apply laboratory findings to real-world scenarios. Future research should concentrate on translating omics-based insights into real-world agricultural settings[28].

3. Proposed Methodology

Integrating proteomics and metabolomics provides a powerful approach for managing plant diseases [29]. The proposed methodology entails systematic steps for identifying, analysing, and applying proteomic and metabolomic data to improve plant disease control strategies. This methodology aims to understand disease mechanisms, identify biomarkers, and create disease-resistant plants.

3.1. Study Design

3.1.1 Objectives

Determine how different proteins and metabolites respond to plant pathogens. Discover molecular mechanisms governing plant-pathogen interactions. Create biomarkers for early disease detection. Develop strategies for breeding disease-resistant plant varieties.

3.1.2 Experimental Plants and Pathogens

Plant Selection: Select representative plant species that are susceptible to the target pathogens.

Pathogen Selection: Choose pathogens relevant to the crops being studied, such as bacteria, fungi, and viruses.

3.3. Sample Collection and Preparation.

3.3.1. Sample collection

Healthy Controls: Gather tissue samples from healthy, untreated plants.

Infected Samples: Take tissue samples from infected plants at various stages of disease progression.

3.**3.2.Samplepreparation**

3.3.2.1 Proteomics:

To perform proteomics, homogenise plant tissues in lysis buffers with protease inhibitors. Protein concentration can be quantified using methods such as the Bradford assay. Use trypsin or other photolytic enzymes to digest proteins [30].

3.3.2.2Sample preparation.

3.4.Proteomics Analysis

3.4.1.Protein Separation and Identification.

Protein Separation: Use SDS-PAGE or isoelectric focussing (IEF) to separate proteins.

Proteins can be analysed using mass spectrometry (MS) techniques such as LC-MS/MS (liquid chromatography-tandem mass spectrometry) or MALDI-TOF.

Protein Identification: Use database searching and bioinformatics tools (such as Mascot and Sequest) to identify proteins and determine their functional annotations[31].

3.4.2.Data Analysis.

Quantitative Analysis: Use software tools (such as MaxQuant and Proteome Discoverer) to quantify protein expression levels. Differential Expression Analysis: Use statistical tools (such as DESeq2 and EdgeR) to identify proteins that are differentially expressed between healthy and infected samples.

Functional Analysis: To understand the biological significance of differentially expressed proteins, use pathway enrichment analysis tools such as KEGG or GO (Gene Ontology) [32].

3.5 Integration of Proteomic and Metabolic Data

3.5.1 Data Integration

Omics Data Fusion: Integrate proteomic and metabolomic data to gain a comprehensive understanding of plant responses. Use multi-omics integration tools (such as Mix Omics and OmicsNet) for this purpose. Network analysis entails building and analysing molecular networks and regulatory pathways affected by disease.

3.5.2 Biomarker Discovery.

Biomarker Identification: Determine which proteins and metabolites are consistently associated with disease states and could be used as biomarkers.

Validation: Test biomarkers on independent plant populations and in field trials [33].

3.5.3 Applications and Implementations

3.5.4 Diagnostic Tools: Create diagnostic assays based on biomarkers discovered for early disease detection and monitoring.

Field Testing: Diagnostic tools should be tested in real-world settings to determine their effectiveness.

3.5.5.Resistant breeding

Marker-Assisted Selection: Use identified biomarkers and associated genes in

marker-assisted breeding programs to create disease-resistant plant varieties. Conduct field trials to determine the effectiveness of resistant varieties.

3.5.6 StressManagement.

Stress Management Strategies: Create strategies based on metabolomic profiles to improve plant resilience, such as using biostimulants or optimising agronomic practices [34].

3.6 Quality Control and Validation.

3.6.1 Replication and reproducibility.

3.6.2 Experimental Replication: Make sure biological and technical replicates are included in experimental designs to validate results.

Reproducibility refers to testing methodologies across multiple laboratories and conditions to ensure their robustness and reliability.

3.6.3 Data Validation.

Cross-validation involves using additional analytical techniques or independent datasets to validate findings.

3.6.4 Field Validation: Confirm laboratory findings in real-world agricultural settings to ensure their practical application.

4. Classifying Proteomics and Metabolomics in Plant Disease Management

Proteomics and metabolomics provide distinct but complementary perspectives on plant disease management. Classifying their roles and applications can aid in understanding their contributions and maximising their use in plant disease management. Here's a detailed classification of proteomics and metabolomics in this context.

4.1 Types of Proteomic Approaches Quantitative proteomics:

Protein abundance is quantified using peak intensities rather than labelling. Isotope Labelling (e.g., SILAC, TMT, iTRAQ): Uses isotopic tags to compare protein levels between samples.

4.2 Qualitative proteomics involves identifying proteins in a sample using mass spectrometry (MS) and database searches.

4.2.1Post-Translational Modifications (PTM):

Phosphoproteomics is the study of how phosphorylation events affect protein function.

Ubiquitinomics investigates ubiquitination and its role in protein degradation.

4.2.2. Applications for Plant Disease Management

4.2.2.1 Pathogen Response Analysis:

Defence Proteins: Detects proteins involved in defence mechanisms, such as

pathogenesis-related proteins.

Signalling Pathways: Examines proteins in signalling pathways activated during pathogen infection (e.g., MAPK pathways).Biomarker Discovery for Disease Biomarkers are proteins that act as early indicators of disease onset or progression.

Resistance Mechanisms:

Resistance Proteins: Researches proteins associated with resistance traits to aid in the development of resistant plant varieties.

Functional proteomics: Functional Characterisation investigates the functional roles of proteins in disease resistance and susceptibility.

4.3 Metabolomic Approach Types

4.3.1 Quantification of Particular Metabolites: Targeted Metabolomics focuses on a predetermined group of metabolites associated with particular pathways.

4.3.2Untargeted Metabolomics: Comprehensive Metabolite Analysis: Recognises and measures an extensive array of metabolites without requiring prior knowledge of their existence.

4.3.3 Semi-Targeted Metabolomics: Broad Spectrum Analysis: Consists of focused examination of specific metabolite classes in a more comprehensive framework.

4.4. Utilisations in the Management of Plant Diseases

Identification of alterations in metabolite levels linked to pathogen infection is possible through metabolic profiling: disease-associated metabolites.

Pathway analysis: associates modified metabolites with particular metabolic routes that play a role in plant defence.

Discovering metabolites that can act as biomarkers for early disease detection and diagnosis is known as "biomarker discovery" or "diagnostic metabolites."

Stress Response: Stress Metabolites: Researches alterations in plant stress response-related metabolite profiles to support stress mitigation techniques.

Mechanisms of Resistance:

Defence Metabolites: Researches metabolites implicated in resistance mechanisms, aiding in the creation of hardy plant cultivars.

4.5 Integration and Comparative Analysis

4.5.1. Evaluation by Comparison

4.5.1.2 Complementary Insights: Metabolomics provides details on alterations in metabolism, whereas proteomics offers insights into the expression and function of proteins.

Pathway Integration: The interplay between proteins and metabolites in disease processes can be uncovered by combining proteomic and metabolomics data.
Proteomics and metabolomics are categorised in relation to plant disease management, which emphasises their different functions and possible overlaps. While metabolomics offers information on metabolic alterations and stress responses, proteomics concentrates on the identification and functional characterisation of proteins involved in disease responses. Combining these methods allows for the creation of sophisticated disease management plans and provides a thorough understanding of plant-pathogen interactions.

4. Results and Discussion

Plant responses to diseases can be fully understood through the use of proteomics and metabolomics. Researchers can identify biomarkers, develop disease management strategies, and learn more about the molecular mechanisms underlying plant-pathogen interactions by analysing changes in protein expression and metabolite profiles. The main conclusions from recent research in these areas are outlined and discussed in this section.

5.1 Using proteomics to manage plant diseases.

5.1.1. Principal Discoveries

5.1.1.1 Expression of Different Proteins

Research has revealed that plants infected with pathogens exhibit elevated expression of pathogenesis-related (PR) proteins, including PR1, PR2, and PR5. According to Jwa et al. (2013), these proteins are essential for both local defence mechanisms and systemic acquired resistance (SAR).

5.1.1.2 Defence Signalling Proteins: Pathogen infection has been shown to upregulate proteins involved in signalling pathways, such as mitogen-activated protein kinases (MAPKs) and protein kinases (Kumar et al., 2014).

5.1.1.3. Changes Made After Translation

Phosphorylation: According to phosphoproteomics, immune response activation depends on phosphorylation events. For instance, signalling pathways that improve plant resistance are triggered by the phosphorylation of particular proteins (Meng et al., 2013).
Ubiquitination: Ubiquitinomics has demonstrated that ubiquitination influences the length and strength of immune responses by facilitating the breakdown of disease-resistant protein (Jiang et al., 2014).

6.2 Discussion

6.2.1 Understanding Mechanisms

Proteomics has yielded significant insights into the molecular mechanisms

underlying resistance to plant diseases. The complex network of defence responses triggered upon pathogen attack is highlighted by the identification of upregulated PR proteins and signalling molecules. New methods for boosting plant resistance can be developed with guidance from an understanding of these mechanisms.

6.2.2 Identification of Biomarkers

The identification of biomarkers for the purpose of early disease detection has been made easier by proteomic data. As an example, certain PR proteins or signalling molecules that are reliably linked to disease states can function as markers of the onset of the disease.

6.2.3 Breeding for Resistance

Important proteins linked to resistance traits have been identified through proteomic analysis. Programs for marker-assisted breeding can use this information to create crops that are more resistant to disease. For instance, choosing plants with higher concentrations of specific defence proteins may result in the emergence of more hardy cultivars.

7. Using Metabolomics to Manage Plant Diseases

7.1.1.Analysis of Metabolism

Pathogen-Induced Metabolites: Salicylic acid (SA) and jasmonic acid (JA), which are involved in both local and systemic defence responses, are two examples of metabolites that have been found through metabolomics studies to increase in response to pathogen infection (Mazzoni et al., 2015).

Secondary Metabolites: It has been discovered that in response to pathogen stress, flavonoids, phenolics, and phytoalexins accumulate and strengthen the plant's defence system (Wang et al., 2019).

7.1.2. Processes of Metabolism

Stress Response Pathways: Metabolomics has identified modifications to important metabolic pathways that are essential for the synthesis of defence compounds, such as the phenylpropanoid pathway (Kim et al., 2021).

Antioxidant Defence: Elevated levels of antioxidants, including glutathione and ascorbic acid, have been noted, suggesting improved mechanisms for the response to oxidative stress (Kollist et al., 2019).

7.2.1. Understanding Metabolism

A thorough understanding of the metabolic alterations plants undergo in reaction to pathogens is offered by metabolomics. Understanding how plants respond to and cope with disease stress is aided by the identification of important metabolites involved in defence responses.

7.2.2. Apps for Diagnosis

Early disease detection diagnostic tools may be developed as a result of the identification of particular metabolites linked to disease states. Plants that are infected can be identified using the metabolic fingerprints of the disease even before symptoms become apparent.

7.2.3. Managing Stress

Developing strategies for stress management is made possible by an understanding of the changes in metabolite profiles. Plant resilience can be increased, for instance, by using biostimulants that increase the synthesis of metabolites linked to defence.

8. Combining Metabolomics and Proteomics

8.1. Consolidated Knowledge

8.1.1.Comprehensive Analysis of Plant Reactions

A more comprehensive picture of plant responses to pathogens can be obtained by combining proteomic and metabolomic data. According to Tzin et al. (2017), integrated analyses show a correlation between alterations in metabolite levels and changes in protein expression, which sheds light on the regulatory networks controlling plant defence.

8.1.2. Improved Finding of Biomarkers

Biomarkers that are more trustworthy for disease detection and resistance evaluation can be found using integrative approaches. Scientists can create more reliable diagnostic and selection tools by establishing connections between proteins and metabolites linked to different disease states.

8.2. Difficulties and Prospects

8.2.1.Complexity of Data

The volume and complexity of data can make it difficult to integrate data from various omics platforms. Sophisticated computational instruments and bioinformatics techniques are needed for efficient management and interpretation of integrated datasets.

8.2.2. Utilising Field Data

It is still difficult to apply research findings from lab experiments to real-world situations. To guarantee the practical utility of biomarkers and strategies, future research should concentrate on validating them in actual agricultural settings.

8.2.3. Developments in Technology

Plant disease understanding and management will be improved by ongoing developments in omics technologies, such as enhanced sensitivity, resolution, and data analysis tools.Proteomics and metabolomics offer complementary

perspectives on the control of plant diseases. Proteomics provides insights into defence mechanisms and possible biomarkers by revealing changes in protein expression and post-translational modifications. By identifying variations in metabolite profiles, metabolomics contributes to the development of diagnostic tools and an understanding of metabolic responses. Combining the two methods yields a thorough understanding of plant-pathogen interactions and helps in the creation of efficient disease management plans. To fully realise the potential of these technologies in agriculture, future research should concentrate on addressing issues with field application and data complexity.

9. Conclusion

Proteomics and Metabolomics in Plant Disease Management: These advanced "omics" technologies offer innovative strategies for managing plant diseases by understanding the molecular mechanisms of plant-pathogen interactions.

Improved Knowledge of Plant Pathology:

Proteomics: Assists in the identification and measurement of proteins implicated in plant defence mechanisms, providing insight into the protein-level responses of plants to pathogen assaults.

Metabolomics: Offers an in-depth analysis of metabolites, which are the byproducts of biological activities. This facilitates comprehension of the metabolites and biochemical pathways involved in plant defence.

FindingBiomarkers:

Plant diseases can be linked to specific biomarkers that can be found using both proteomics and metabolomics. These biomarkers enable prompt and accurate intervention by aiding in the early detection and diagnosis of diseases.

Creation of Resistance Types:

Breeders can create new plant varieties that are more disease-resistant by comprehending the proteins and metabolites involved in plant resistance. This encourages sustainable agriculture and lessens the need for chemical treatments.

Targeted Illness Treatment:

The development of targeted treatments is made possible by the knowledge gained from proteomics and metabolomics. To increase resistance against infections, for example, particular proteins or metabolites that improve plant immunity can be targeted.

Tracking and Evaluating the Success of Treatment:

By examining alterations in the proteome and metabolome of treated plants, these technologies can be used to track the efficacy of disease management plans and make sure that the techniques being used are working.

Proteomics and metabolomics are critical for improving plant disease management because they provide a detailed molecular understanding of plant-pathogen interactions. These technologies help to identify disease biomarkers, develop resistant plant varieties, and implement targeted and effective disease management strategies. By incorporating proteomics and metabolomics into plant disease management, we can create more sustainable and resilient agricultural practices.

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CHAPTER – 5

PRECISION AGRICULTURE: ICT TOOLS IN DISEASE MONITORING

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Abstract
Precision agriculture leverages Information and Communication Technology (ICT) tools to enhance the monitoring, diagnosis, and management of crop diseases with high accuracy and efficiency. The integration of tools such as remote sensing, geographic information systems (GIS), drones, mobile-based apps, and artificial intelligence enables real-time disease surveillance, early warning systems, and data-driven decision-making. These technologies help in identifying disease outbreaks at an early stage, applying site-specific treatments, and reducing the overuse of agrochemicals. ICT-based disease monitoring not only improves crop health and yield but also contributes to sustainable and cost-effective farming practices by optimizing resource use and minimizing environmental impact.
Keywords: Precision agriculture, ICT tools, Disease monitoring, Remote sensing, Sustainable farming

Introduction:

Overview of Precision Agriculture

Precision Agriculture (PA) is an innovative farming approach that utilizes advanced technologies to optimize field-level management regarding crop farming. By integrating tools such as GPS, remote sensing, GIS, and data analytics, precision agriculture enables farmers to assess and respond to intra-field variability in real time. This targeted approach leads to better resource utilization, increased yields, cost savings, and environmental sustainability.

Importance of Disease Monitoring in Agriculture

Plant diseases pose a significant threat to global food security, often leading to substantial yield losses and economic hardship for farmers. Early detection and effective monitoring of diseases are crucial for timely intervention and management. Traditional methods of disease surveillance often rely on manual scouting, which is time-consuming and less efficient,

particularly in large-scale farms. Integrating systematic disease monitoring helps in preventing the spread of infections, reducing pesticide use, and ensuring the health and productivity of crops.

Role of ICT in Enhancing Disease Monitoring

Information and Communication Technologies (ICT) play a pivotal role in revolutionizing disease monitoring in agriculture. The use of mobile applications, satellite imagery, drones, machine learning, and IoT devices allows real-time data collection, analysis, and dissemination. ICT tools enable farmers and agricultural experts to receive timely alerts, access diagnostic tools, and implement appropriate control measures quickly. These technologies contribute to creating smarter, more resilient agricultural systems that can adapt to biotic stressors efficiently.

1. Remote Sensing:

Use of Satellites and Drones in Disease Detection

Remote sensing technologies, particularly satellites and drones, have transformed how plant diseases are detected and monitored. Satellites provide large-scale, continuous observation of agricultural fields, making it possible to track disease progression over time and across vast areas. Drones, on the other hand, offer high-resolution imagery at the field level and can be deployed more frequently for localized assessments. These tools enable early identification of stress symptoms such as discoloration, canopy changes, and leaf wilting, which are often indicative of disease presence.

Spectral Imaging for Disease Identification

Spectral imaging, including multispectral and hyperspectral techniques, is a powerful tool for detecting plant diseases. These methods capture images across different wavelengths of light, many of which are not visible to the human eye. Healthy plants reflect and absorb light differently than diseased plants. By analyzing vegetation indices like NDVI (Normalized Difference Vegetation Index), remote sensing systems can detect anomalies associated with disease stress before visible symptoms appear. This early detection capability allows for timely and targeted interventions.

Case Studies on Remote Sensing Applications

Several successful applications of remote sensing in agriculture illustrate its effectiveness in disease monitoring:

- **Wheat Rust in South Asia**: Satellite imagery combined with ground surveillance helped forecast and track the spread of wheat rust, enabling early warning systems across India and Pakistan.

- **Grape Powdery Mildew in California**: Drone-based hyperspectral imaging was used to detect powdery mildew in vineyards, leading to precise fungicide application and reduced chemical use.
- **Rice Blast in Southeast Asia**: Remote sensing data integrated with weather and crop models allowed the prediction and monitoring of rice blast outbreaks, improving decision-making for disease management.

2. Geographic Information Systems (GIS):

Mapping Disease Outbreaks and Spread

GIS is a powerful tool for visualizing and analyzing the spatial distribution of plant diseases. By plotting disease occurrences on a map, GIS enables the identification of hotspots, patterns of spread, and regions at higher risk. This spatial mapping supports better surveillance and helps extension workers and farmers make informed decisions regarding quarantine, treatment, or crop rotation. Temporal mapping also allows tracking of disease progression over time.

Integration of GIS with Remote Sensing Data

Combining GIS with remote sensing enhances disease monitoring capabilities by linking spatial data (e.g., disease incidence) with spectral and environmental data collected from satellites or drones. For instance, areas showing abnormal NDVI values from remote sensing can be cross-referenced with GIS-based disease occurrence records to confirm disease presence. This integration allows for precise targeting of interventions, improved monitoring of large areas, and supports real-time decision-making.

Predictive Modeling for Disease Risk Assessment

GIS also plays a critical role in developing predictive models for disease risk assessment. By incorporating various spatial layers such as climate data, soil type, crop type, and historical disease outbreaks, GIS enables the simulation of disease scenarios. These models help forecast potential outbreaks and assess the vulnerability of different regions under various environmental conditions. For example, disease risk maps generated through GIS can guide the timing and location of fungicide applications, reducing unnecessary chemical use and costs.

3. Mobile Applications in Agricultural disease monitoring

Farmer-Friendly Apps for Disease Reporting and Diagnosis

Mobile applications have become essential tools for farmers to detect, report, and manage plant diseases easily. These apps often feature user-friendly interfaces, local language support, and image-based diagnostics. Farmers can

upload photos of affected crops and receive instant feedback on possible diseases and recommended treatments. Apps like Plantix**,** CropIn**,** and Kisan Suvidha are widely used for such purposes, enabling even smallholder farmers to access expert-level diagnostic support from their smartphones.

Real-time Data Collection and Sharing

Mobile apps facilitate the collection and transmission of real-time data from the field to researchers, extension officers, and policy-makers. This includes geo-tagged images, disease incidence reports, and environmental data. Such real-time sharing helps in rapid disease outbreak detection and quick response planning. Additionally, mobile-based platforms often allow crowdsourcing of data, improving the accuracy and scale of disease surveillance.

Case Studies of Successful Implementations

Several initiatives around the world have shown the impact of mobile apps in improving plant health management:

- **PlantVillage Nuru (Sub-Saharan Africa):** This AI-powered app assists farmers in diagnosing diseases in crops like cassava and maize using phone cameras, even in offline mode. It has significantly reduced diagnosis time and increased awareness of disease management strategies.
- **mKisan (India):** This government-backed app delivers personalized alerts, weather forecasts, and plant protection advisories to farmers, including disease outbreak notifications.
- **eLocust3m (FAO):** Though focused on pest management, this app allows frontline workers to report locust swarms via smartphones, and the same approach has been adapted for disease reporting in other crops.

4. Machine Learning and AI in plant disease monitoring

Algorithms for Disease Prediction and Classification

Machine learning (ML) algorithms are increasingly being used to predict disease outbreaks and classify plant diseases based on various data inputs. Supervised learning models such as Support Vector Machines (SVM), Random Forest, and Convolutional Neural Networks (CNNs) are commonly employed to identify disease types from image and environmental datasets. Time-series models like LSTM (Long Short-Term Memory) are also used to predict the likelihood of disease outbreaks based on historical and climatic data. These algorithms help in automating decision-making and improving the accuracy of disease forecasting systems.

Automated Image Analysis for Symptom Detection

AI, especially deep learning, has shown remarkable success in image-based disease detection. Using high-resolution images of crop leaves or fruits, AI models can detect visual symptoms like spots, blights, wilting, or discoloration. CNNs are particularly effective in analyzing such image data, distinguishing between disease symptoms and other stress factors (e.g., nutrient deficiency or drought). These models can be embedded into mobile apps or drone-based platforms, enabling rapid and automated diagnosis without requiring expert intervention in the field.

Challenges in Developing Robust AI Models

Despite their potential, several challenges exist in developing reliable AI models for plant disease management:

- **Data Scarcity and Quality**: High-quality, annotated datasets are limited, especially for less common crops and diseases.
- **Environmental Variability**: Disease symptoms may vary due to light, angle, weather, or growth stage, affecting model accuracy.
- **Generalization**: Many models fail when applied to different regions or crop varieties due to overfitting or lack of diverse training data.
- **Field Deployment**: Translating lab-trained models into field-ready solutions that work on low-power devices or in offline mode remains a hurdle.

5. Challenges and Future Directions:

Data Privacy and Security Concerns

As disease monitoring becomes increasingly digital, large volumes of farm-level data—often including geo-location, crop health, and farmer identities—are collected and transmitted. This raises serious concerns about data privacy, ownership, and security. Without clear policies or farmer consent, there is a risk of misuse or unauthorized access to sensitive agricultural data by third parties, including agri-tech companies or marketers. Ensuring encrypted data transmission, secure cloud storage, and transparent data-sharing agreements are essential for building trust and protecting user rights.

Infrastructure and Connectivity Issues

Despite the rapid digitalization in agriculture, poor internet connectivity, lack of electricity, and limited smartphone access remain major barriers in many rural areas. Remote sensing tools and mobile apps require reliable infrastructure to function effectively. In addition, the digital literacy of farmers is often low, which limits their ability to fully benefit from ICT-based disease monitoring solutions. Addressing these challenges requires

investment in rural broadband, user training, and development of low-bandwidth and offline-compatible tools.

Future Trends in ICT Tools for Disease Monitoring

Looking ahead, several promising trends are expected to reshape how plant diseases are monitored and managed:

- **Edge Computing and IoT Devices**: Smart sensors and edge devices that process data locally will allow real-time disease alerts without relying on cloud access.
- **AI-Powered Predictive Platforms**: Integration of AI with weather and crop models will improve accuracy in predicting disease outbreaks before symptoms appear.
- **Blockchain for Data Integrity**: Blockchain technology can ensure secure, tamper-proof recording of field data and disease records, enhancing transparency and traceability.
- **Integration with Digital Advisory Services**: Future ICT tools will be embedded into broader digital platforms that combine disease diagnostics with agronomic advice, weather updates, and market access

Conclusion:

Summary of Key Points

This overview has highlighted the transformative potential of Information and Communication Technologies (ICT) in enhancing plant disease monitoring. Precision agriculture, supported by tools like remote sensing, GIS, mobile applications, and AI, enables early detection, real-time reporting, and accurate prediction of disease outbreaks. These technologies not only improve response time but also reduce crop losses, optimize chemical usage, and support informed decision-making.

The Role of ICT in Sustainable Agriculture

ICT plays a vital role in achieving sustainable and climate-resilient agriculture. By minimizing input waste, improving disease control efficiency, and empowering farmers with timely information, digital tools help increase productivity while conserving natural resources. The integration of smart technologies into farming practices fosters transparency, traceability, and adaptability—key pillars of long-term agricultural sustainability.

Recommendations for Stakeholders

To fully realize the benefits of ICT in disease monitoring, coordinated efforts are required from all stakeholders:

- **Policy Makers**: Formulate clear data privacy regulations and support ICT infrastructure development in rural areas.
- **Researchers and Developers**: Focus on creating robust, locally adaptable AI models and mobile tools that work in low-connectivity environments.
- **Extension Services and NGOs**: Train farmers in digital literacy and promote the adoption of ICT tools through community-based models.
- **Private Sector**: Invest in affordable, farmer-centric innovations and ensure ethical use of collected agricultural data.
- **Farmers**: Actively participate in digital platforms, report field conditions accurately, and adopt smart farming practices.

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CHAPTER – 6

NANOFUNGICIDES: FORMULATION AND DELIVERY MECHANISMS

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Abstract
Nanofungicides represent a cutting-edge advancement in plant disease management, utilizing nanotechnology to improve the formulation, efficiency, and targeted delivery of antifungal agents. Unlike conventional fungicides, nanofungicides enhance solubility, stability, and controlled release, resulting in reduced dosage, minimized environmental contamination, and improved pathogen specificity. Common formulations include nanoparticles of metals (e.g., silver, copper), nanoemulsions, and polymer-based nanocarriers, which are designed to penetrate fungal structures or plant tissues effectively. Delivery mechanisms involve foliar sprays, seed treatments, or soil applications that ensure precise targeting and sustained action. The integration of nanofungicides into crop protection strategies offers a promising route toward sustainable agriculture with reduced chemical inputs and improved plant health outcomes.
Keywords: Nanofungicides, Targeted delivery, Nano formulation, Plant disease management, Controlled release

1. Introduction

Overview of fungicides and their role in Agriculture: Fungicides are chemical or biological agents used to control fungal pathogens that cause diseases in crops, leading to significant yield losses. They play a crucial role in agriculture by protecting plants from infections like blights, rusts, and mildews, ensuring healthy growth and maximizing productivity. Fungicides can be categorized into systemic, contact, and protectant types, each with specific modes of action. Their application is essential for managing crop diseases, especially in high-value crops like fruits, vegetables, and cereals. However, reliance on fungicides also raises concerns about environmental impact, resistance development, and human health risks.

Limitations of conventional fungicides: Conventional fungicides face several limitations that hinder their effectiveness and sustainability. Over

time, many plant pathogens develop resistance to these chemicals, reducing their efficacy and requiring higher doses or more frequent applications. This not only increases production costs but also contributes to environmental pollution, affecting soil health and non-target organisms, including beneficial insects and aquatic life. Additionally, the persistence of chemical residues in food products raises concerns about human health. Regulatory restrictions on certain fungicides due to their toxicological profiles further complicate their use, driving the need for alternative, more sustainable disease management strategies

Introduction to nanotechnology in Agriculture: Nanotechnology in agriculture represents a transformative approach to improving crop protection, nutrient delivery, and environmental sustainability. By manipulating materials at the nanoscale, researchers can develop innovative solutions such as nanofertilizers, nanopesticides, and nanofungicides. These nano-based tools offer enhanced efficiency and targeted delivery, reducing the amount of chemicals needed while minimizing environmental impact. Nanotechnology also enables the creation of smart systems that can respond to environmental stimuli, optimizing the timing and release of active ingredients. As global agriculture faces challenges like climate change and pest resistance, nanotechnology presents promising avenues for more sustainable and effective farming practices.

Definition of Nanofungicides: Nanofungicides: Fungicides formulated using nanotechnology, involving the use of nanomaterials or nanoparticles to enhance the delivery and effectiveness of the active ingredients.

Nanocarriers: Tiny particles, often ranging from 1 to 100 nanometers in size, that encapsulate or bind to fungicides to improve their stability, delivery, and controlled release.

Significance of Nanofungicides

1. **Enhanced Efficacy:** Increased surface area of nanoparticles allows for better interaction with target pathogens, potentially improving fungicide effectiveness even at lower doses.
2. **Targeted Delivery:** Nanofungicides can be designed for targeted delivery, reducing off-target effects and minimizing damage to beneficial organisms.
3. **Controlled Release:** Nanotechnology enables controlled and sustained release of fungicides, ensuring prolonged protection and reducing the need for frequent applications.

4. **Reduced Environmental Impact:** Lower dosages and targeted delivery reduce environmental contamination and the potential for harmful residues.
5. **Overcoming Resistance:** Nanofungicides may help in managing resistance by providing new modes of action or enhancing the effectiveness of existing fungicidal compounds.
6. **Improved Stability:** Encapsulation of fungicides within nanoparticles can protect them from degradation due to environmental factors like UV light or pH changes.
7. **Integration with Precision Agriculture:** Nanofungicides can be combined with precision agriculture techniques for more accurate and efficient disease management, contributing to sustainable farming practices.

2. Formulation Strategies

2.1. Nanocarriers in Fungicide Delivery

2.1.1 Types of Nanocarriers

Liposomes:

- Spherical vesicles with a lipid bilayer, capable of encapsulating both hydrophilic and hydrophobic fungicides.
- Advantages: Biocompatibility, ability to merge with plant cell membranes for better delivery.
- Applications: Used for the controlled release of fungicides, improving stability and reducing toxicity.

Nanoparticles:

- Solid colloidal particles, typically made from materials like metals (e.g., silver, zinc oxide), polymers, or silica.
- Advantages: High surface area-to-volume ratio, potential for surface modification to enhance targeting.
- Applications: Enhance fungicide uptake by pathogens, provide slow release, and increase penetration through plant cuticles.

Nanoemulsions:

- Fine oil-in-water or water-in-oil emulsions with droplet sizes in the nanometer range.
- Advantages: Increased solubility of hydrophobic fungicides, improved stability, and controlled release.

- Applications: Used to improve the dispersion of fungicides in aqueous environments, enhancing bioavailability and efficacy.

2.1.2 Functionalization of Nanocarriers for Fungicide Delivery

Surface Modification:

- Functionalizing the surface of nanocarriers with ligands, antibodies, or peptides to target specific pathogens or plant tissues.
- Improves specificity, reduces off-target effects, and enhances the interaction with fungal pathogens.

Controlled Release Mechanisms:

- Incorporation of stimuli-responsive materials that release fungicides in response to environmental triggers (e.g., pH, temperature).
- Provides a sustained and controlled release, reducing the frequency of fungicide applications.

2.2. Synthesis of Nanofungicides

2.2.1 Physical Methods

Milling:

- Mechanical reduction of particle size using high-energy ball mills or similar equipment.
- Produces nanoparticles with uniform size distribution, suitable for enhancing fungicide solubility and bioavailability.

High-Pressure Homogenization:

- Forcing a fungicide solution through a narrow gap at high pressure to create nanoscale droplets or particles.
- Often used to produce nanoemulsions or to reduce the size of particles in suspensions.

2.2.2 Chemical Methods

Sol-Gel:

- A process that involves the transition of a system from a liquid "sol" (colloidal solution) into a solid "gel" phase.
- Used for synthesizing metal oxide nanoparticles that can be loaded with fungicides, offering high surface area and stability.

Co-Precipitation:

- Simultaneous precipitation of multiple components from a solution, leading to the formation of composite nanoparticles.
- Allows for the incorporation of fungicides into the matrix of nanoparticles, enhancing protection and controlled release.

2.2.3 Biological Methods

Biosynthesis Using Microorganisms:

- Utilizing bacteria, fungi, or yeast to produce nanoparticles through biological processes.
- Provides an eco-friendly synthesis method, reducing the need for harsh chemicals or energy-intensive processes.

Biosynthesis Using Plants:

- Employing plant extracts as reducing agents to synthesize nanoparticles from metal salts.
- Offers a green alternative for nanoparticle production, with the added benefit of plant-derived bioactive compounds that may enhance fungicidal activity.

2.3. Encapsulation Techniques

2.3.1 Lipid-Based Systems

Liposomes:

- Encapsulation of fungicides within lipid bilayers, offering protection and controlled release.
- Can be engineered to fuse with plant cell membranes, enhancing the delivery of active ingredients directly to target sites.

Solid Lipid Nanoparticles (SLNs):

- Fungicides encapsulated within solid lipid matrices, providing controlled release and improved stability.
- Useful for delivering hydrophobic fungicides in a controlled manner.

2.3.2 Polymer-Based Systems

Polymeric Nanoparticles:

- Encapsulation of fungicides within biodegradable polymers like PLGA (poly(lactic-co-glycolic acid)).

- Offers controlled release, protection from degradation, and reduced environmental impact.

Nanogels:

- Cross-linked polymer networks that can swell in response to environmental conditions, releasing the encapsulated fungicide.
- Useful for stimuli-responsive release based on pH or temperature changes.

2.3.3 Hybrid Systems

Inorganic-Organic Hybrids:

- Combining organic polymers with inorganic nanoparticles (e.g., silica, metal oxides) for enhanced stability and controlled release.
- Can offer the benefits of both organic and inorganic systems, such as biocompatibility and high mechanical strength.

Multilayered Nanocarriers:

- Encapsulation of fungicides within multiple layers of different materials (e.g., lipid-polymer combinations) to achieve sequential release profiles.
- Provides enhanced protection and allows for the release of multiple active ingredients in a controlled manner.

3. 3. Delivery Mechanisms

3.1. Targeted Delivery

3.1.1 Passive vs. Active Targeting

Passive Targeting:

- Relies on the natural distribution of nanofungicides through the plant's vascular system or the external environment.
- **Mechanism**: Nanofungicides are absorbed by plant tissues through natural processes, such as diffusion or root uptake, and may accumulate in specific areas based on size, charge, and hydrophobicity.
- **Applications**: Often used for broad-spectrum applications where targeted delivery is less critical.

Active Targeting:

- Involves the modification of nanofungicides to specifically bind to target sites, such as fungal pathogens or specific plant tissues.

- **Mechanism**: Surface modifications, such as the attachment of ligands, antibodies, or peptides, enable nanofungicides to selectively bind to specific receptors on fungal cells or plant tissues.
- **Applications**: Enhances the precision of fungicide application, reducing off-target effects and improving efficacy against specific pathogens.

3.1.2 Surface Modifications and Ligand Attachment

Surface Modifications:

- Techniques to alter the surface properties of nanocarriers, such as coating with polymers, lipids, or surfactants, to improve stability, biocompatibility, and targeting ability.
- **Examples**: Coating nanoparticles with polyethylene glycol (PEG) to reduce immune recognition or adding chitosan to enhance adhesion to plant surfaces.

Ligand Attachment:

- Attaching specific molecules, such as antibodies, peptides, or small molecules, to the surface of nanocarriers to enhance their affinity for target sites.
- **Examples**: Functionalizing nanocarriers with lectins that bind to fungal cell wall components, or using peptides that target specific plant receptors to enhance uptake in infected tissues.
- **Benefits**: Increases the specificity of fungicide delivery, reduces required dosage, and minimizes harm to beneficial organisms.

3.2. Controlled Release

3.2.1 Stimuli-Responsive Release

pH - Responsive Systems:

- Designed to release fungicides in response to pH changes in the environment, such as acidic conditions in infected tissues.
- **Mechanism**: Nanocarriers are engineered to degrade or swell in response to specific pH levels, releasing the encapsulated fungicide.
- **Applications**: Targeted release in acidic environments, such as fungal infection sites, minimizing damage to healthy plant tissues.

Temperature-Responsive Systems:

- Release fungicides in response to temperature fluctuations, often triggered by the microenvironment around infection sites.
- **Mechanism**: Nanocarriers may contain materials that undergo phase transitions or structural changes at specific temperatures, triggering the release of fungicides.
- **Applications**: Useful in climates where temperature variations are significant, ensuring fungicide release when conditions are optimal for pathogen growth.

Enzyme-Responsive Systems:

- Release fungicides in response to the presence of specific enzymes produced by fungal pathogens or the plant during infection.
- **Mechanism**: Nanocarriers are designed with enzyme-cleavable linkages that degrade in the presence of specific enzymes, releasing the fungicide directly at the infection site.
- **Applications**: Targeted release in the presence of fungal enzymes, improving specificity and reducing the impact on non-target organisms.

3.2.2 Sustained Release Mechanisms

Diffusion-Controlled Release:

- Fungicides are gradually released from nanocarriers as they diffuse through the carrier matrix over time.
- **Mechanism**: Nanocarriers are designed with porous or degradable materials that allow the slow release of fungicides, maintaining effective concentrations over an extended period.
- **Applications**: Reduces the frequency of fungicide applications, providing prolonged protection against pathogens.

Degradation-Controlled Release:

- Release of fungicides is controlled by the degradation rate of the nanocarrier material.
- **Mechanism**: Biodegradable polymers or other materials slowly break down in the plant environment, releasing the fungicide as the carrier degrades.

- **Applications**: Ensures a consistent release rate, tailored to the lifecycle of the pathogen or the duration of the crop growth cycle.

Osmosis-Controlled Release:

- Utilizes osmotic pressure differences to regulate the release of fungicides from nanocarriers.
- **Mechanism**: Nanocarriers are designed with semi-permeable membranes that allow water to enter, creating pressure that gradually pushes the fungicide out.
- **Applications**: Suitable for controlled delivery in soil or hydroponic systems, where water availability is consistent.

3.3. Uptake and Translocation in Plants

3.3.1 Interaction with Plant Surfaces

Cuticular Penetration:

- Understanding how nanofungicides penetrate the waxy cuticle of leaves or stems, which acts as a barrier to pathogen entry and chemical absorption.
- **Mechanism**: Nanocarriers can be designed to enhance penetration through cuticular cracks, stomata, or by interacting with surface lipids.
- **Applications**: Improving the uptake of fungicides in aerial parts of plants, ensuring effective delivery to target tissues.

Root Uptake:

- The process by which nanofungicides are absorbed through the roots and transported via the xylem or phloem to other parts of the plant.
- **Mechanism**: Nanocarriers can enhance root absorption by interacting with root exudates or modifying the rhizosphere environment.
- **Applications**: Effective for soil-applied fungicides targeting root-borne pathogens or systemic diseases.

3.3.2 Movement within Plant Tissues

Xylem Translocation:

- The movement of nanofungicides through the xylem, facilitating upward transport from roots to leaves and other aerial parts.

- **Mechanism**: Nanocarriers designed to remain stable in the xylem sap, ensuring consistent delivery to target sites.
- **Applications**: Important for systemic control of foliar pathogens and diseases affecting the upper parts of plants.

Phloem Translocation:

- Movement of nanofungicides through the phloem, enabling downward transport to roots and other storage tissues.
- **Mechanism**: Nanocarriers can be engineered to move with the flow of phloem sap, ensuring delivery to sites of active growth or pathogen colonization.
- **Applications**: Targeting phloem-associated pathogens or delivering fungicides to developing tissues.

3.3.3 Effect on Systemic Resistance

Induction of Systemic Acquired Resistance (SAR):

- How nanofungicides may enhance a plant's natural defense mechanisms, leading to broad-spectrum resistance against various pathogens.
- **Mechanism**: Nanocarriers can be designed to deliver elicitors or signaling molecules that trigger SAR pathways, boosting the plant's immune response.
- **Applications**: Reducing the need for repeated fungicide applications by enhancing the plant's inherent ability to resist infection.

Priming of Defense Responses:

- Nanofungicides may "prime" plant tissues, making them more responsive to subsequent pathogen attacks.
- **Mechanism**: Low doses of fungicides or associated compounds delivered by nanocarriers may activate defense genes, preparing the plant for faster and stronger responses to infection.
- **Applications**: Enhancing the effectiveness of fungicides and reducing overall chemical inputs in integrated pest management (IPM) strategies.

4. Efficacy and Safety Considerations

4.1. Efficacy against Plant Pathogens

Comparative Studies with Conventional Fungicides

Comparative efficacy studies involve evaluating novel fungicides or biopesticides against standard chemical fungicides such as mancozeb, carbendazim, chlorothalonil, or metalaxyl. Parameters assessed include disease incidence reduction, severity index, yield improvement, and phytotoxicity.

1. Disease Suppression

In field and greenhouse trials, natural products like neem-based formulations, Bacillus subtilis, Trichoderma spp., and essential oil-based fungicides have shown comparable or even superior control over certain pathogens such as *Alternaria solani* (early blight in tomato), *Sclerotinia sclerotiorum* (stem rot in sunflower), and *Fusarium oxysporum* (wilt diseases).

2. Yield and Quality Improvement

Although conventional fungicides often show rapid disease suppression, prolonged usage can lead to residue accumulation and resistance development. In contrast, bio-fungicides, though slower in action, tend to improve soil health, enhance systemic resistance, and lead to better fruit or grain quality in the long term.

3. Resistance Management

Many conventional fungicides have a single-site mode of action, making them prone to resistance development. Alternative fungicides, especially microbial-based ones, typically operate through multiple mechanisms (e.g., competition, antibiosis, induced resistance), reducing the likelihood of resistance development.

Spectrum of activity

The term "spectrum of activity" refers to the range of pathogens a fungicide can effectively control. A broad-spectrum fungicide is effective against multiple classes of fungi—oomycetes, ascomycetes, and basidiomycetes—while narrow-spectrum fungicides target specific groups.

1. Broad-Spectrum Fungicides

- **Conventional Examples:** Mancozeb and chlorothalonil are widely used for their broad-spectrum activity against foliar pathogens.
- **Alternative Examples:** Extracts of *Azadirachta indica*, *Allium sativum*, and essential oils (e.g., thyme, clove) have shown inhibitory effects on a wide array of pathogens, including *Botrytis cinerea*, *Pythium spp.*, and *Rhizoctonia solani*.

2. Target-Specific Activity

Some alternative fungicides exhibit high efficacy against specific pathogens:

- **Pseudomonas fluorescens** is highly effective against *Fusarium* and *Rhizoctonia* species.
- **Trichoderma harzianum** is particularly active against soil-borne pathogens like *Sclerotium rolfsii*.

3. Synergistic Effects

Recent studies also show that combining bio-fungicides with low doses of conventional fungicides can lead to synergistic effects, improving disease control and reducing chemical inputs.

4.2. Environmental and Health Impacts

Toxicity to Non-Target Organisms

Fungicides—whether synthetic or nano-based—can impact a variety of non-target organisms, including beneficial soil microbes, pollinators, aquatic life, and even vertebrates.

- **Pollinators and Beneficial Insects:** Some fungicides may negatively affect the behavior, reproduction, or survival of bees and natural enemies like ladybird beetles or parasitoids. Nanoparticles such as silver or zinc oxide, though effective against pathogens, have been shown to induce oxidative stress in insects.
- **Soil Microorganisms:** Trichoderma, Rhizobium, and mycorrhizal fungi are crucial for soil health. Overuse of chemical fungicides or high doses of nanomaterials can disrupt microbial diversity, enzyme activity, and nutrient cycling.
- **Aquatic Ecosystems:** Runoff containing fungicides or nanoparticles can contaminate water bodies, posing risks to fish, amphibians, and plankton. Persistence of certain compounds like chlorothalonil or nano-copper in aquatic habitats leads to bioaccumulation and trophic-level transfer.
- **Human Exposure:** Applicators and farm workers may be exposed to fungicides through dermal contact or inhalation. Although nanomaterials are used at lower dosages, their small size allows deeper tissue penetration, raising concerns of cytotoxicity and genotoxicity.

Degradation and persistence in the environment

The fate of fungicides in the environment is influenced by their chemical structure, formulation, and application method.

- **Degradability:** Many conventional fungicides have long half-lives and break down slowly in soil or water, resulting in persistent residues. In contrast, bio-fungicides degrade more readily but may have shorter protective periods.
- **Nano-formulations:** These are designed to enhance stability and control release, but their persistence may increase depending on their coating and reactivity. Nanoparticles can bind to soil components or accumulate in plant tissues, where their breakdown mechanisms are still not fully understood.
- **Environmental Accumulation:** Repeated applications without proper rotation or degradation can lead to fungicide buildup in soil, reducing microbial diversity and potentially affecting subsequent crop growth.
- **Volatilization and Leaching:** Some fungicides volatilize into the atmosphere or leach into groundwater. This not only reduces their effectiveness but also contributes to broader environmental contamination.

4.3. Regulatory Aspects

Current regulations for nanomaterials in agriculture

India: The Central Insecticides Board and Registration Committee (CIBRC) has started considering guidelines for nano-formulations. However, specific criteria for registration and risk assessment are still evolving.

European Union: The EU requires explicit labeling and safety documentation for nano-enabled pesticides under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). The EFSA (European Food Safety Authority) provides guidance for the risk assessment of nanomaterials in food and agriculture.

United States: The EPA has issued specific guidelines for the registration of nanopesticides, emphasizing environmental fate and human exposure. Nanomaterial-containing products undergo case-by-case evaluation based on their unique properties.

International Harmonization: Organizations like OECD are working toward harmonizing nanomaterial risk assessment and data-sharing protocols.

- **Risk assessment and safety evaluation.**

  Risk assessment of fungicides and nano-formulations involves several key steps:

  Hazard Identification: Determining potential toxic effects on human and environmental health.

  Exposure Assessment: Evaluating routes and levels of exposure for applicators, consumers, and ecosystems.

  Dose-Response Assessment: Establishing the relationship between exposure level and adverse effects.

  Risk Characterization: Integrating hazard and exposure data to estimate the probability and severity of risk.

5. Future Perspectives

As the demand for sustainable and effective plant disease management continues to grow, nanotechnology offers promising avenues for innovation. The future of nanofungicides lies in developing smart, efficient, and environmentally benign products that align with the principles of precision agriculture and integrated disease management.

5.1 Advances in Nanofungicide Research

Emerging Nanomaterials for Fungicide Delivery

Recent years have witnessed rapid development in the synthesis of novel nanomaterials for controlled and targeted fungicide delivery. These include:

- Metallic nanoparticles (silver, copper, zinc oxide) with intrinsic antifungal properties.
- Polymeric nanoparticles, liposomes, and nanogels for encapsulating active ingredients.
- Clay-based nanocarriers like halloysite and montmorillonite for slow-release formulations.
- Such materials enhance fungicide stability, bioavailability, and uptake, while reducing application frequency and environmental load.

Integrating Nanotechnology with Precision Agriculture

Nanotechnology holds great promise in advancing precision agriculture by enabling:

- Smart delivery systems, such as stimuli-responsive nanoparticles that release fungicides in response to pH, temperature, or pathogen presence.
- Nano-enabled sensors that detect early disease outbreaks or monitor environmental parameters.
- Site-specific application, minimizing off-target impacts and optimizing input use through variable rate technologies.

This integration can significantly improve the timing, dosage, and localization of fungicide application, resulting in higher efficiency and reduced waste.

5.2 Challenges and Opportunities

Technical Challenges in Large-Scale Production

Despite laboratory-scale success, scaling up the production of nanofungicides remains challenging due to:

- High cost of raw materials and synthesis.
- Difficulties in ensuring uniform particle size and stability.
- Lack of robust protocols for formulation and storage under field conditions.

Public Perception and Acceptance

There is growing public concern about the safety of nanomaterials in food and agriculture. Key issues include:

- Perceived risks of nanoparticles entering the food chain.
- Lack of transparent labeling and regulatory clarity.
- Mistrust due to limited communication from industry and researchers.
- Building trust through education, transparent risk assessments, and stakeholder engagement is essential.

Bridging the Gap between Research and Commercialization

While academic research has generated a wealth of nanofungicide technologies, only a few have reached the market. This is due to regulatory uncertainty, limited field validation, and insufficient industry partnerships. Strengthening interdisciplinary collaboration and promoting public-private investment can accelerate the transition from lab-scale innovation to field-ready solutions.

6. Conclusion

The development of nanofungicides represents a significant advancement in sustainable plant disease management. These innovative formulations offer

enhanced efficacy, targeted delivery, and reduced environmental impact compared to conventional fungicides. Throughout this chapter, the comparative performance of nanofungicides, their broad-spectrum activity, and lower toxicity to non-target organisms have been emphasized, highlighting their potential for integrated disease management systems.

Nanomaterials such as silver, copper, zinc oxide, and biodegradable polymers are paving the way for more precise and controlled fungicide applications. While concerns remain regarding environmental persistence, safety, and public perception, ongoing research and regulatory development are gradually addressing these challenges. Integration with precision agriculture tools further enhances the appeal of nanofungicides by enabling site-specific, real-time disease control.

In summary, nanofungicides hold tremendous promise in transforming agricultural practices. By improving disease control efficiency while minimizing ecological and health risks, they can contribute to more resilient and environmentally conscious farming systems. With continued interdisciplinary research, thoughtful regulation, and informed public dialogue, nanotechnology can be harnessed to secure the future of plant protection and global food security.

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CHAPTER 7

NANOTECHNOLOGY FOR TARGETED DISEASE CONTROL

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Abstract
Targeted disease control (TDC) in the agricultural sector represents a strategic initiative aimed at mitigating crop losses caused by pathogens and other factors. The role of nanotechnology in TDC is significant, as it has the potential to revolutionize both the detection and treatment of various plant diseases. This technology is poised to enhance sustainable and productive agricultural practices, thereby addressing the increasing global population and food requirements. Nanobiosensors are instrumental in identifying biotic and abiotic stresses in plants prior to their impact on crop yield, while nanoparticles can enhance the efficiency of agrochemicals, reducing their environmental footprint. Nanofibers are emerging as innovative materials, leading to a diverse range of nanoparticle-based formulations, including fertilizers, herbicides, insecticides, and fungicides. Nevertheless, the advancement of nanotechnology applications is impeded by insufficient understanding of nanoparticle transformations and the interactions between nanoparticles and macromolecules within crops.

Key words: Agriculture, Nanotechnology, Nanoparticles, pathogens, TDC.

Introduction

Agriculture serves as a fundamental science focused on the scientific cultivation of crops to address global hunger. With the world population growing at an unprecedented rate and projected to reach 10 billion by 2050 [1], enhancing crop productivity has become a paramount concern for nations worldwide. The primary goals of food production are to enhance both quality and quantity. Nevertheless, various biotic and abiotic factors significantly contribute to losses in global food production. Common biotic factors include pathogens such as bacteria, fungi, and viruses, which pose challenges during crop cultivation. Abiotic factors, such as drought, soil

salinity, extreme temperatures, and cold conditions, represent significant constraints on crop production in terms of both quality and quantity. The impact of these challenges is expected to intensify due to climate change and the increasing demands of a rising human population. Since the recognition of the potential damage caused by the pathogens, humans have implemented numerous strategies to reduce their impact. To reduce food loss and increase profitability, practices such as chemical fertilizer, genetic modification, and integrated pest management are employed [2]. Despite these efforts, crops continue to face threats from a range of bacterial, fungal, and viral pathogens. It is estimated that globally, 20–40% of total agricultural output is compromised due to pest infestations, with plant pathogens accounting for 16% of the yield loss [3]. In India, the potential loss of crops due to diseases, pests, and weeds is estimated to be around 15–25% of the annual food production.

Nanotechnology presents a promising avenue for the transformation of agricultural sectors. It facilitates the understanding of the biochemical pathways in crops by enhancing traditional methods used to assess environmental challenges and applying these insights to improve production [4]. When compared to environmentally sustainable technologies and agricultural biotechnology, nanotechnology offers the potential for a more significant and rapid impact on all elements of the agricultural value chain, yielding synchronized benefits that encompass legal, ethical, and environmental considerations [5]. The anticipated application of nanoscale agrochemicals, including nanofertilizers, nanopesticides, nanobiosensors, and nanoformulations, has revolutionized conventional agricultural practices, rendering them more sustainable and efficient. Various applications of nanotechnology in agriculture include wastewater treatment, remediation of contaminated soil, and enhancing crop productivity through the use of sensors designed to detect pathogens [6].

2. Nanotechnology

Nanotechnology in the science, that deals with creation of material at nanoscale (1 to 100 nanometre (nm)) by manipulating atoms and molecules. Richard Feynman, an American physicist, is considered the father of nanotechnology. The term “Nanotechnology” was introduced by a Japanese scientist, Norio Taniguchi in 1974. The advent of modern nanotechnology can be traced back in 1981, when the introduction of the scanning tunnel

microscope enabled researcher to observe and manipulate single atoms. Later in the beginning of 20th century, the nanotechnology has bloomed, nearly all developing countries established initiatives focused on nanotechnology research. Nanotechnology, despite its nomenclature, has significantly transformed various industries worldwide.

2.1 Nanoparticles

A particle of matter with diameter of 1 to 100 nm are called nanoparticle or ultrafine particle. Nanoparticles possess a unique property when compared the same particle at macroscale this is due to their size, minuscule dimension and significant surface area. When particle reaches the nanoscale, the periodic boundary conditions the crystalline structure are disrupted, particularly when the characteristic length scale is comparable to or smaller than the de Broglie wavelength or the wavelength of light [8].

2.2 Types of nanoparticles

Nanoparticles can be classified into various types according to their size, shape and material characteristics. Nanoparticle are broadly classified into two category based on nature of particles they created as organic and inorganic nanoparticles. The organic nanoparticles are present in nature and it also be synthesized, they include dendrimers, liposome, polymersome, capsule, vesicles and polymer conjugates [9]. The inorganic nanoparticles are made up of non-carbon based molecule such as fullerenes, quantum dots, metal based nanoparticle example gold nanoparticles. The inorganic nanoparticles have more advantage then organic nanoparticles because they are non-toxic, hydrophilic biocompatibility, and highly stable nature [10]. Furthermore, nanoparticles can be classified as hard, such as titanium (titanium dioxide), silica (silica dioxide) particles or as soft like liposome, vesicles and nanodroplets. The classification often influenced by their intended application, whether for diagnostic purposes or therapeutic purposes, or may relate to their production method.

Based on structure nanoparticles are classified into two types, nanocystalline and nano-structured. Nanocrystalline particles are the drug particles developed at nanosize without carrier. These nanocrystals have more advantages than novel drugs because of increased solubility, high bioavailability due to rapid absorption, increased surface area, minimal side effect and high cost-effective. Nano-structured are the carriers used as drug delivery system for targeted drug delivery. Nano-structured materials are

further classified into polymer-based, non-polymeric, and lipid-based nanoparticles. Polymer-based nanoparticles include dendrimers, nanoparticles, micelles, nanogels, protein nanoparticles and drug conjugates. Non-polymeric nanoparticles are carbon nanotubes, nanodiamond, metallic nanoparticles, quantum dotes, and silica-bases nanoparticles.

2.3. Technical aspects of developing and screening nanoparticles

The development of nanoparticles has three main approaches: physical, chemical and biological approaches. The physical approach is the top-down nanoparticle manufacturing method, were the bulk materials are chopped and sculpted to nanometer-scale dimension. Mechanical milling, electrospinning, laser ablation, sputtering, electron explosion, sonication, lithography kind of methodologies are followed in top-down approach. On other hand the chemical and biological approaches follows bottom-up methodology, in which the atoms and molecules are combined to create nanoscale particles. In this type of approach chemical vapor deposition, sol-gel process, co-precipitation, molecular condensation, hydrothermal, green/ biology synthesis with in living system methods are followed [11]. Both top-down and bottom-up methods are used in developing nanomedicines. New synthetic methods such as emulsion polymerization technique, amphiphile self-assembly, and polymerization-induced self-assembly in bottom-up approach and lithographic templating and 3D printing in top-up approach are used in present-days [12].

The screening of developed nanoparticles required sophisticated and highly sensitive instrumentation setup. These techniques are highly specific in order the screen the nanoparticles characters such as size, diameter, surface area, bonding, functional group, and composition [11]. Most importantly atomic force microscope and scanning tunneling microscope are used to visualize and manipulate individual atoms [13,14]. Ultra centrifugation and liquid chromatography techniques are used to separate and purify synthesized nanoparticles from reaction solution [15, 16]. Optical study of material and to determine the synthesis of nanoparticle are done using ultraviolet-visible and Fourier transform infrared spectroscopy [17, 18]. For structural characterization several techniques used that include X-ray diffraction, dynamic light scattering, zeta potential, Raman spectroscopy, and Auger electron spectroscopy.

3. Nanotechnology for Targeted Disease Control

Targeted disease control (TDC) in agriculture sector is the strategical approach to prevent crop loss due to pathogens and other causes. Focusing on disease control requires the establishment of precise objectives aimed at reducing, eliminating, or eradicating diseases. Nanotechnology plays a vital role in TDC, which possesses the capability to transform both detection and treatment of various plant diseases. In TDC early detection and effective treatment are most important strategies in controlling any kind of plant diseases. Nanoparticles offer various methods for controling plant diseases, including: antimicrobial agents, fertilizers, production of defense compounds, immune system enhancement, carriers, rapid pathogen identification and biosensor-based management.

3.1 Application of nanotechnology in diagnosing plant diseases

The prompt identification and diagnosis of plant pathogens are essential for TDC. Various immunological, serological, and nucleic acid-based detection assays have been developed to ensure accurate identification of plant pathogens. In the last twenty years, significant efforts have been directed towards creating methods for diagnosing and monitoring plant infections through biochemical assays that utilize specific proteins, toxins, ELISA, nucleic acid probe technologies, and PCR amplification of nucleic acid sequences [19]. Though these biochemical assays have several drawbacks like complex sampling techniques, expensive infrastructure, limited number of plant diseases can be effectively identified, inadequate for on-site disease detection in agricultural fields. Consequently, there is a pressing need for the development of cost-effective techniques to enhance the accuracy and speed of plant pathogen diagnostics. The advancement of nanomaterials has enabled the creation of swift and highly sensitive diagnostic techniques that can be employed in the field for the early detection of plant diseases [20]. These nanomaterials have been integrated into molecular assays to enhance both sensitivity and selectivity in identifying plant pathogens or disease indicators, including nucleic acids, proteins, toxins, and carbohydrates.

3.1.1 Nanobiosensors

Nanobiosensors are the device that use nanoparticle to monitor specific stresses of plants and detect them in advance even at small scale. Nanobiosensors are utilized in both field settings and laboratory environments for analytical purposes. The integration of nanoscience,

computing, biology, and electronics is enabling researchers to create nanobiosensors with enhanced sensing capabilities. The essential characteristics of nanobiosensors, they should be cost-effective, biocompatible, non-toxic, and portable; easy maintain standard storage conditions; highly specific, minimal reactivity; and precise, accurate, and reproducible [21]. Nanoparticles, including silver (Ag), gold (Au), and metal oxide, are frequently utilized as nanobiosensors. Gold nanoparticle that were functionalized with single-stranded oligonucleotides to detect the genomic DNA of *Ralstonia solanacearum*, the pathogen responsible for potato bacterial wilt. Additionally, it has been utilized in the detection of the plant bacterial pathogen *Pantoea stewartii* [22]. Silver nanorods have been applied to identify the plant pathogen *Phytophthora ramorum* from actual samples via surface-enhanced Raman spectroscopy [23]. DNA biosensor has been developed by utilizing zinc oxide nanoparticle/chitosan nanocomposites to detect *Trichoderma harzianum*, a soil-borne fungus. Quantum dots, due to its low toxicity and excellent biocompatibility make them particularly suitable for application as nanobiosensors in microorganism detection [24].

3.2 Application of nanotechnology in disease control.

The next level of TDC is to generate efficient drugs to eradicate these plant diseases. Nanoparticle itself serve as an efficient drug or drug carrier that kill pathogens and provide nutrients. In recent decades, several nanoprotectants, and nanocarrier are produced by researchers and they show a great impact of TDC in agriculture.

3.2.1 Nanoparticle as protectant

Nanoparticles can serve as effective antimicrobial agents on their own and have demonstrated efficacy against a variety of soil-borne pathogens. They can be applied to various parts of the plant, including soil, seeds, roots, and foliage, to offer protection against pests and pathogens, including fungi, bacteria, and viruses. These nanoparticles are capable of penetrating the plant system, where they either act directly against the pathogens or function as elicitor molecules that stimulate local and systemic defence mechanisms in plants. Among the most extensively researched metallic nanoparticles are gold, silver, titanium oxide, zinc oxide, and copper oxide, all of which are recognized for their antifungal, antibacterial, and antiviral properties [25]. Biogenically synthesized silver nanoparticles shows antifungal character against various phytopathogens, including *Sclerotium rolfsii*, *Rhizoctonia*

solani, Fusarium oxysporum, and Sclerotinia sclerotiorum [26]. Another finding indicated that copper oxide nanoparticles notably diminished the occurrence of tomato bacterial wilt disease, which is caused by the soil-borne bacterium *Ralstonia solanacearum* [27].

3.2.2 Nanoparticle in drug delivery system as carrier

Nanoparticles are frequently employed as carriers to entrap, encapsulate, absorb, or attach active molecules, thereby facilitating the development of effective agricultural formulations. Silica, chitosan, solid lipid nanoparticles, and layered double hydroxide nanoparticles are among the most common carriers used in plant disease management [28]. Silica nanoparticles can be synthesized with precision regarding their size, shape, and structure, rendering them highly effective delivery vehicles. Chitosan nanoparticles itself shows antifungal activity and it can also be transformed into carrier for drug delivery due to their hydrophobic characteristics, allowing them to adhere effectively to the epidermis of leaves and stems. This property enhances contact time and promotes the uptake of bioactive molecules [29]. Similarly, solid lipid nanoparticles, and layered double hydroxide nanoparticles are used as carrier for drug delivery of pesticides, insecticides, fungicides, herbicides and RNA interference-inducing molecules.

4. Challenges of nanotechnology application in agriculture.

The advancements reported in the realm of nanotechnology within agriculture are increasingly encouraging, with a multitude of patents and commercial products already in existence. However, the integration of these products into practical agricultural applications remains challenging. This domain required multidisciplinary teams possessing expertise in fields such as chemistry, physics, biology, and agronomy. The behaviour of nanomaterials can vary significantly plants to plants [30]. Though it is effective and acceptable at large scale sectors, small farmers may be disinclined to adopt these innovations [31]. One of the primary obstacles in the utilization of various nanodevices in agriculture is the additional financial burden they may impose on farmers. The implementation of nanobiosensors presents further complications, as tailored methods must be developed for their application on crops; in many instances, they may prove ineffective or prohibitively expensive, particularly in the case of non-invasive electronic systems applied to leaves, unless utilized in perennial crops such as fruit

trees [32]. To overcome these challenges, it is essential to prioritize research aimed at field applications.

5. Conclusion:

The human race is completely depending on agriculture for life sustainability, but many factors like pathogens, global warming, soil infertility and so on had great impact on crop production. TDC of plant diseases is the important strategy to identify root cause of plant diseases and efficient treatment. The application of nanotechnology in the management of plant diseases holds significant potential. Nanoparticles serve not only as antimicrobial agents but also as effective delivery systems for active compounds aimed at inhibiting phytopathogens. The primary benefits of nanoparticles, in comparison to traditional products and methods, include enhanced effectiveness, reduced initial costs, and lower toxicity to non-target organisms. Nevertheless, the environmental behavior of nanoparticle remains poorly understood, and there are concerns regarding their potential bioaccumulation within food chains. Therefore, it is essential to thoroughly assess the environmental risks associated with their widespread agricultural use. Consequently, the adoption of alternative materials that are non-toxic, biocompatible, and biodegradable, such as biopolymer-based nanoparticles, along with biosynthesis techniques for nanoparticle, is recommended. Furthermore, to mitigate environmental impact, further research should focus on optimizing the size, shape, and surface functionalization of nanoparticle to enhance their efficacy.

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CHAPTER 8

ENVIRONMENTAL AND SAFETY ASPECTS OF NANOMATERIALS IN AGRICULTURE

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Abstract
This chapter delves into the multifaceted implications of employing nanomaterials in agriculture, focusing on environmental and safety concerns. The advent of nanotechnology in agriculture offers promising enhancements in crop production, pest control and nutrient delivery. However, the unique properties of nanomaterials pose potential risks to ecosystems and human health. The chapter explores the advantages and applications of nanomaterials in agriculture such as improved pesticides efficacy and soil remediation. It then, crucially examines the environmental impact, addressing issues like nanoparticle accumulation in soil and water, potential toxicity to non-target organisms and the disruption of microbial communities. Additionally, it discusses the human health risks associated with exposure to engineered nanomaterials including inhalation and ingestion pathways. Regulatory frameworks and safety guidelines are reviewed, highlighting the current gaps and the need for comprehensive risk assessment methodologies. Through a balanced analysis of both opportunities and hazards, this chapter aims to provide a nuanced understanding on the safety of nanomaterials integration into agriculture ensuring sustainable development.

Keywords: Nanoparticles, soil, environment, engineered, crops.

Introduction

The integration of nanotechnology into agricultural practices represents a groundbreaking advancement, promising significant improvements in productivity, efficiency and sustainability. Nanomaterials with their unique properties such as high surface area to volume ratio, enhanced reactivity and tuneable physical and chemical characteristics, have found diverse

applications in agriculture [1]. From nano-fertilizers and nano-pesticides to sensors for precision farming and soil remediation techniques, the potential benefits of nanomaterials are immense. They offer innovative solutions for enhancing crop yields, reducing chemical inputs and mitigating environmental impacts traditionally associated with conventional farming methods. However, the introduction of nanomaterials into the agricultural ecosystem is not without challenges and concerns. They very attributes that make nanomaterials advantageous can also pose significant risks to the environment and human health. Understanding these risks is crucial for developing safe and sustainable agricultural practices [2]. Nanomaterials can interact with plants, soil, water and air in complex ways, potentially leading to unintended consequences. For instance, their persistence and bioaccumulation in the environment may affect soil health, aquatic systems and non-target organisms, including beneficial insects and microorganisms essential for ecosystem balance [3]. This chapter aims to provide a comprehensive overview of the environmental and safety aspects of nanomaterials in agriculture. It will explore the current applications and emerging trends in the use of nanomaterials, highlighting both the advantages and potential risks. The discussion will delve into the mechanisms of nanoparticle interaction with agricultural systems, examining their fate, transport and ecological impact. Furthermore, the chapter will address human health implications, considering exposure routes and toxicity. Regulatory frameworks and safety guidelines will also be reviewed, identifying existing gaps and proposing strategies for effective risk management.

Applications of Nanomaterials in Agriculture

Nanotechnology has introduced a paradigm shift in agricultural practices, offering innovative solutions to enhance productivity, efficiency and sustainability. The unique properties of nanomaterials such as their small size, large surface area and enhanced reactivity enable a range of applications that can significantly benefit the agricultural sector.

Nano-fertilizers: Nano-fertilizers are designed to improve nutrient delivery and uptake by plants. Traditional fertilizers often suffer from leaching and volatilization, leading to nutrient loss and environmental pollution. Nano-fertilizers release nutrients in a controlled manner ensuring efficient absorption by plant roots. This targeted delivery reduces the number of

fertilizers needed, minimizes environmental impact and enhances crop yields [4].

Nano-pesticides: Nano-pesticides offer a promising alternative to conventional chemical pesticides. Encapsulation of active ingredients in nanoparticles can protect them from degradation and enhance their stability. This allows for lower dosages and more effective pest control with reduced environmental contamination. Additionally, nano-pesticides can be engineered to release their active ingredients in response to specific environmental triggers further increasing their efficacy and safety [5].

Nano-sensors for precision farming: Precision farming relies on real time data to optimize agricultural practices. Nano-sensors detect soil moisture nutrients levels and pest presence with high sensitivity and accuracy. These sensors provide farmers with valuable information, enabling precise application of water, fertilizers and pesticides. This not only improves crop management but also conserves resources and minimizes environmental impact [6].

Soil remediation techniques: Contaminated soils pose a significant challenge to agriculture. Nanomaterials can be used for soil remediation by adsorbing and degrading pollutants. Iron oxide nanoparticles can remove heavy metals from soil, while titanium dioxide nanoparticles can degrade organic pollutants through photocatalysis. these nanomaterials offer efficient and cost-effective solutions for restoring soil health and ensuring safe agricultural production [7].

Figure 1: Applications of nanotechnology in agriculture

Beyond the primary applications nanomaterials are being explored for various other uses in agriculture (**Figure 1**). These include enhancing seed germination, improving plant resistance to stress and facilitating the delivery of genetic material for crop improvement [8]. Nanomaterials can also be incorporated into packaging materials to extend the shelf life of agricultural produce by inhibiting microbial growth and reducing spoilage [9]. The integration of nanomaterials in agriculture holds great promise for addressing the challenges of modern farming. By enhancing the efficiency of inputs, improving crop yields and reducing environmental impact, nanotechnology can contribute to sustainable agricultural practices [10]. However, it is crucial to carefully evaluate the potential risks associated with nanomaterials to ensure their safe and responsible use in the agricultural ecosystem [10].

Environmental impact of nanomaterials

The incorporation of nanomaterials into agricultural practices offers significant benefits but it also raises critical environmental concerns. Nanomaterials due to their unique properties interact with the environment in complex and often unpredictable ways. Understanding these interactions is essential for assessing their overall environmental impact.

Fate and transport of nanoparticles in soil: When nanomaterials are applied to agricultural fields their small size and high surface area can lead to extensive dispersion in soil. They can bind to soil particles, move through

soil layers and potentially reach groundwater systems. The mobility and persistence of nanoparticles depend on their chemical composition, surface coatings and environmental conditions. Like in metal-based nanoparticles such as silver or zinc oxide can dissolve and release metal ions, which may have different environmental behaviors compared to their nanoparticulate form [11].

Effects on soil health and fertility: Nanomaterials can influence soil health by affecting its physical, chemical and biological properties. While certain nanomaterials may improve soil properties and promote plant growth, others might have adverse effects. For instance, high concentrations of metal nanoparticles can be toxic to beneficial soil microorganisms, altering microbial diversity and activity. This disruption can impair nutrient cycling, organic matter decomposition and overall soil fertility. Move over, nanoparticles can affect the soil's physical structure, potentially impacting water retention and aeration [12].

Impact on aquatic systems: Water from agricultural fields can transport nanomaterials top nearby water bodies, where they may pose risks to aquatic ecosystems. In water, nanoparticles can aggregate, settle or remain suspended depending on their properties and environmental conditions. Aquatic organisms, ranging from microorganisms to fish, can be exposed to these particles. Studies have shown that certain nanoparticles can be toxic to aquatic life, causing oxidative stress, cellular damage and behavioural changes. The bioaccumulation of nanoparticles in aquatic food webs further complicates their ecological impact [13].

Interaction with non-target organisms: Nanomaterials designed for agricultural use can inadvertently affect non-target organism, including beneficial insects, plants and wildlife. For example, nano-pesticides may harm pollinators like bees, which are crucial for crop pollination. Additionally, nanoparticles can be taken up by non-target plants, potentially entering the food chain and affecting herbivores and predators. The extent of these impacts depends on the type, concentration and exposure duration of the nanomaterials [14].

Regulatory and safety considerations: Addressing the environmental impact of nanomaterials requires robust regulatory frameworks and safety guidelines. Current regulations often lag technological advancements creating gaps in risk assessment and management. Comprehensive

environmental impact assessments including life cycle analysis are essential to evaluate the benefits and risks of nanomaterials [15].

Critical issues of occupational risk in the nano-agricultural field

The advent of nanotechnology in agriculture brings innovative solutions but also raises significant occupational health risks for workers involved in the production, handling and application of nanomaterials. These risks are attributed to the unique properties of nanoparticles which can lead to enhanced reactivity and potential toxicity [16].

Exposure pathways: Agricultural workers can be exposed to nanomaterials through inhalation, dermal contact and ingestion. Inhalation is a primary concern as nanoparticles can become airborne during manufacturing, mixing or spraying processes. Due to their small size, these particles can penetrate deep into the respiratory system, potentially causing pulmonary inflammation and other respiratory diseases. Dermal exposure can occur when workers handle nanomaterials without proper protective equipment leading to skin absorption and possible systemic effects. Ingestion might happen through hand-to-mouth activities or contaminated food and water sources in the workplace [17].

Toxicological concerns: Nanoparticles can exhibit different toxicological profiles compared to their bulk counterparts. The high surface area to volume ratio increases their chemical reactivity which can result in oxidative stress, cellular damage and inflammatory responses. Metal based nanoparticles like silver, titanium dioxide can generate reactive oxygen species leading to cytotoxicity. Chronic exposure to such nanoparticles may contribute to long term health issues including respiratory diseases, cardiovascular problems and potentially carcinogenic effects.

Lack of awareness and training: A significant issue in the nano-agricultural fields is the lack of awareness and proper training among workers regarding the potential hazards of nanomaterials. Many agricultural workers may not be fully informed about the risks or the safety protocols necessary to mitigate exposure. This gap in knowledge can lead to inadequate use of personal protective equipment and improper handling practices [18].

Inadequate regulatory frameworks: Existing occupational health and safety regulations often do not specifically address the unique challenges posed by nanoparticles. This regulatory gap can result in insufficient

protection measures for workers. There is a critical need for comprehensive guidelines and standards that consider the specific properties and risk associated with nanomaterials [19].

Risk management strategies: Effective risk management strategies include the implementation of engineering controls such as proper ventilation systems to reduce airborne nanoparticles and administrative controls like training programs and exposure monitoring. The use of appropriate PPE, including respirators and protective clothing is essential to minimize exposure. Regular health surveillance of workers exposed to nanomaterials can also help in early detection and management of potential health issues [20].

Conclusion

The incorporation of nanomaterials in agriculture presents a dual edged sword offering substantial benefits alongside significant environmental and safety challenges. This chapter has explored the multifaced roles nanomaterials play in enhancing agricultural productivity, efficiency and sustainability while also scrutinizing their potential risks to ecosystems and human health. Nanotechnology enables precise nutrient delivery, effective pest control and innovative soil remediation techniques. Nano-fertilizers and nano-pesticides promise to reduce chemical usage and improved crop yield aligning with sustainable agricultural practices. However, the environmental impact of these nanomaterials cannot be overlooked. Their behaviour in soil, water and air systems is complex and often unpredictable raising concerns about their persistence, bioaccumulation and toxicity. Environmental interactions of nanoparticles such as their fate and transport in soil, potential contamination of aquatic systems and effects on non-target organisms underscore the necessity for comprehensive environmental risk assessments. The disruption of soil health and microbial communities coupled with the bioaccumulation of nanoparticles in aquatic food webs, exemplifies the ecological risks that require thorough investigation and mitigation. Occupational exposure to nanomaterials poses additional safety concerns. Agricultural workers are at risk through inhalation, dermal contact and ingestion of nanoparticles necessitating robust safety protocols and adequate training to mitigate these risks. A balanced integration of nanomaterials into agricultural practices requires a synergistic effort between researchers, policymakers and industry stakeholders. Future research should focus on

elucidating the long-term environmental impacts refining risk assessment models and developing safer nanomaterials. Policymakers must establish comprehensive regulations that safeguard environmental and human health without stifling innovation. By addressing these challenges head on the agricultural sector can harness the benefits of nanotechnology in a manner that promotes sustainability and safety ensuring a resilient and productive future for global agriculture.

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CHPATER 9

NANO-ENCAPSULATION OF BIOLOGICAL CONTROL AGENTS

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Abstract:

The use of biological control agents (BCAs) offers a sustainable alternative to chemical pesticides, enhancing agricultural productivity while minimizing environmental impact. However, the practical application of BCAs is often hindered by their instability and sensitivity to environmental conditions. Nano-encapsulation presents a promising solution to these challenges, providing a protective barrier that enhances the stability, efficacy and controlled release of BCAs. This chapter delves into the principles and methodologies of nano-encapsulation examining the various materials and techniques used to encapsulate BCAs. We explore the advantages of nano-encapsulation in improving the shelf life and bioavailability of BCAs as well as its role in targeted delivery and reduced dosage requirements. The chapter also addresses the challenges and future perspectives in the field including the scalability of nano-encapsulation techniques and regulatory considerations. The chapter aims to provide insights into the potential of nano-encapsulation to revolutionize the use of BCAs in sustainable agriculture, highlighting its significance in achieving enhanced pest control, reduced environmental impact and improved crop yields.

Keywords: Pest control, nanotechnology, sustainable agriculture, crop yield improvement, agrochemical.

Introduction

The increasing demand for sustainable agricultural practices has led to a renewed focus on biological control agents (BCAs) as viable alternatives to chemical pesticides. BCAs which include beneficial microorganisms, viruses and natural substances, offer an eco-friendly approach to pest and disease management, promoting healthier crops and reducing the environmental footprint of agriculture. However, the widespread application of BCAs is often constrained by challenges related to their stability, effectiveness and

environmental sensitivity. These agents can degrade quickly under adverse environmental conditions, lose efficacy over time and require precise delivery mechanism to target pest effectively without harming beneficial organisms [1].

Nano-encapsulation has emerged as a transformative technology addressing these limitations. Bu encasing BCAs within nanostructured materials, nano-encapsulation protects them from environmental degradation, enhances their stability and ensures a controlled and sustained release. Nanocarriers includes liposomes, polymeric nanoparticles and nanogels are engineered to deliver BCAs precisely where they are needed minimizing off-target effects and reducing the required dosage. Such targeted delivery systems are crucial for maintaining ecological balance and maximising the benefits of BCAs [2].

The integration of nanotechnology in agricultural practices represents a significant advancement towards achieving sustainable and efficient pest management. This chapter delves into the underlying principles of nano-encapsulation, the diverse materials and techniques used and the specific benefits they confer to BCAs. By providing a comprehensive overview of recent advancements and future perspectives, this chapter aims to highlight the pivotal role of nano-encapsulation in revolutionizing the use of BCAs ultimately contributing to more sustainable and productive agricultural systems.

Principles of nano-encapsulation

Nano-encapsulation involves the encapsulation of substances such as BCAs within nanoscale carriers to enhance their stability, efficacy and delivery. The principles of nano-encapsulation are rooted in the manipulation of materials at the nanometre scale, typically ranging from 1 to 100 nanometres. This scale allows for precise control over the properties and behaviours of the encapsulated agents. The fundamental principle behind nano-encapsulation is to create a protective barrier around the BCAs which shields them from environmental factors such as degradation, oxidation and moisture. Their barrier is composed of materials like biodegradable polymers, lipids or nanogels which form a nanostructured matrix around the agent. This encapsulation matrix is designed to be biocompatible and non-toxic ensuring that the BCAs can be safely applied in agricultural setting without adverse effects on plants, pests or the environment.

The encapsulation process typically involves techniques such as solvent evaporation coacervation or electrospinning. These methods allow for the formation of nanocarriers with tailored properties including size, shape and surface characteristics. The choice of encapsulation technique and material is crucial in determining the stability, release profile and overall performance of the BCAs. This innovative approach improves the effectiveness and sustainability of pest management strategies contributing to more efficient and environmentally friendly agricultural practices [3].

Materials for Nano-Encpsulation

Materials for nano-encapsulation play a crucial role in determining the effectiveness and efficiency of biological control agents in sustainable agriculture. Various materials are employed for nano-encapsulation, each offering distinct advantages and functionalities that cater to specific agricultural needs. One of the most used materials is biodegradable polymers which include poly (lactic-co-glycolic acid) (PLGA), chitosan and alginate. Liposomes are prominent material that are spherical vesicles composed of lipid bilayers they are highly biocompatible and can encapsulate both hydrophilic and hydrophobic substances, providing versatile delivery. Liposomes protect BCAs from degradation and facilitate targeted delivery to specific sites within plants or pests enhancing the precision and effectiveness of pest control measures [4].

Polymeric nanoparticles such as those made from polycaprolactone and polylactic acid are also widely used in nanoencapsulation. These materials offer excellent mechanical properties stability and the ability to carry high payload of BCAs. Polymeric nanoparticles can be engineered to respond to specific environmental triggers such as pH or temperature changes providing smart delivery systems that release BCAs under optimal conditions. Nanogels which are hydrophilic polymer networks offer another innovative approach. Their high-water content a tunable properties make them suitable for delivering BCAs in aqueous environments ensuring efficient dispersal and uptake by plants. Nanogels can encapsulate a wide range of BCAs and release them in response to environmental stimuli enhancing the adaptability and effectiveness of pest control strategies [5].

The choice of materials for nano-encapsulation is pivotal in enhancing the performance of BCAs. Biodegradable polymers, liposomes polymeric nanoparticles and nanogels each provide unique benefits contributing to the

development of advanced sustainable agricultural practices that maximize pest control efficacy while minimizing environmental impacts.

Methods of Nano encapsulation of biological control agents

Nano encapsulation of biological control agents is a cutting-edge approach that enhances the stability, efficacy and targeted delivery of these agents in agricultural applications. By incorporating nanotechnology, it is possible to overcome many of the limitations associated with conventional biocontrol methods [6]. The methods include:

Chemical methods:

Chemical methods of Nano encapsulation involve the creation of nanoparticles through chemical reactions. One common technique is emulsion polymerization where monomers are polymerised within an emulsified system to form polymeric nanoparticles that encapsulate BCAs. This method allows precise control over particle size and surface characteristics crucial for protecting BCAs and ensuring their controlled release. Sol-gel processes are another approach where a solution transforms from a liquid sol into solid gel phase forming inorganic nanoparticles like silica. These nanoparticles can encapsulate BCAs providing a protective matrix that shields them from environmental stressors while enabling their gradual release.

Physical methods:

Physical methods rely on physical processes to encapsulate BCAs. Spray drying is a widely used technique where a solution containing the BCA and a carrier material is atomized into a hot drying chamber, resulting in the rapid formation of dry nanoparticles. This method is advantageous for its scalability and ability to produce stable powders. Freeze-drying involves freezing the BCA solution and then sublimating the ice under vacuum, leaving dry nanoparticles. This technique is particularly suitable for temperature sensitive BCAs as it minimizes thermal degradation and preserves their biological activity.

Biological methods:

Biological methods utilize biopolymers and naturally occurring materials for encapsulation. Liposomes are spherical vesicles formed from lipid bilayers that can encapsulate both hydrophilic and hydrophobic BCAs. Liposomes are biocompatible and biodegradable, making them an ideal delivery system that can fuse with plant call membranes, releasing the BCAs directly where

needed. Chitosan nanoparticles derived from chitosan biopolymer offer antimicrobial properties and enhance the adhesion and controlled release of BCAs on plant surfaces. These biopolymers are eco-friendly are eco-friendly and align with the principles of sustainable agriculture ensuring minimal environmental impact while providing effective pest and disease management [7].

Combination Techniques:

Combining different encapsulation methods can optimize the efficiency and functionality of the nano-bioformulations. Layer by layer assembly involves the sequential adsorption of oppositely charged polymers around the BCA, creating multilayered nanocapsules. This technique allows for precise control over the release kinetics and protection of the BCAs. Coacervation is another method where a liquid phase containing the BCA separates from a polymer-rich phase followed by cross-linking to form stable nanoparticles. This technique is versatile and can be adapted to encapsulate a wide range of BCAs with varying properties.

Advantages of Nano-encapsulation

They offer several distinct advantages that significantly enhance the application of biological control agents in agriculture. One of the primary benefits is the improvement in the stability of BCAs. Nano-encapsulation protects these agents from environmental factors such as UV radiation, moisture and temperature fluctuations which can degrade their efficacy. By creating a protective barrier nano-encapsulation ensures that BCAs retains their biological activity over extended periods thus extending their shelf life and reducing the frequency of applications. Nano-encapsulation allows for the gradual release of encapsulated agents which can be precisely timed to coincide with pest activity or plant needs. This controlled release maximised the risk of over-application and reduces the overall quantity of BCAs required leading to cost effective and decreased environmental impact [8].

Enhanced bioavailability is another crucial benefit of nano-encapsulation. Encapsulation improves the solubility and dispersibility of BCAs ensuring that they are more readily absorbed by plants and pests. This increased bioavailability enhances the effectiveness of BCAs allowing for more efficient pest management and better crop protection. Additionally, nano-encapsulation supports targeted delivery, which is vital for minimizing off-target effects. By using specific nanocarriers, BCAs can be directed precisely

to the sites where they are needed, reducing the impact on non-target organisms and improving the overall ecological balance. This targeted approach also helps in preserving beneficial microorganisms and natural predators contributing to a more sustainable agricultural system. These benefits collectively contribute to more effective and sustainable pest management practices reinforcing the role of BCAs as a viable alternative to chemical pesticides in modern agriculture [9] (**Figure 1**).

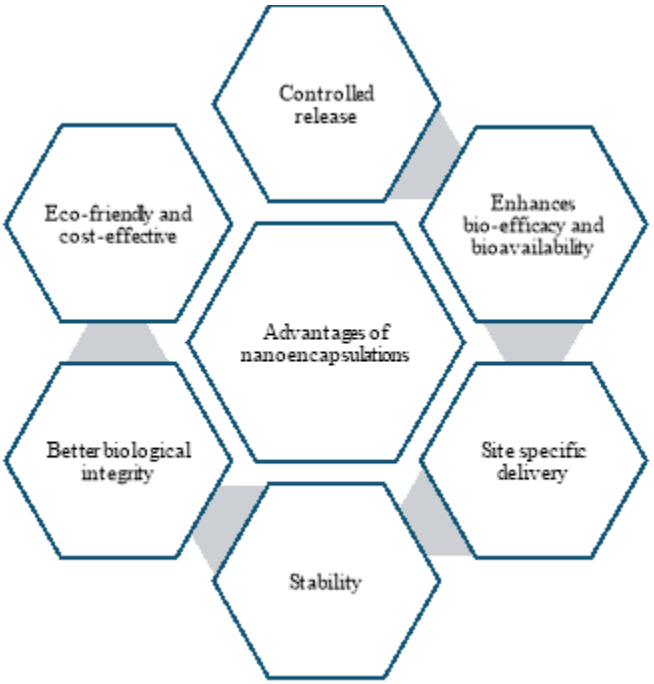

Figure 1: Advantages of nano-encapsulation using biological control agents.

BCAs as mitigative agents of plant biotic stresses

BCAs serve as crucial mitigative agents in managing plant biotic stresses which include pests, diseases and other harmful organisms that negatively affect crop health and productivity. BCAs leverage natural processes and interactions to control these biotic stresses in a more environmentally friendly and sustainable manner compared to conventional chemical pesticides [10].

Pest management

BCAs offer a natural alternative to synthetic pesticides by utilizing natural predators, parasites or pathogens to control pests. In case of ladybugs and lacewings are introduced into crops to feed on aphids and other harmful pests. Parasitic wasps can target and lay eggs in pest insects, effectively

reducing the numbers. Pathogenic microorganisms such as entomopathogenic fungi or bacteria infect and kill pests offering a biological method to mange pest populations. These BCAs are often highly specific to their target's pests, which helps preserve beneficial organisms and maintains ecological balance [11].

Disease management

BCAs also plays a significant role in managing plant diseases caused by fungi, bacteria and viruses. Beneficial microorganisms like *Trichoderma* species are known to supress soil-borne pathogens through mechanisms such as competition for resources, production of antimicrobial compounds and induction of plant defenses. Similarly, *Bacillus subtilis*, a bacterium that acts as biological fungicide by producing substances that inhibit the growth of pathogenic fungi. The use of these BCAs can reduce the incidence and severity of diseases, leading to healthier plants and improved crop yields [12].

Mitigating biotic stresses

In addition to direct pest and disease control, BCAs help mitigate biotic stresses by enhancing plant resilience and promoting plant growth. Certain BCAs can induce systemic resistance in plants making them more resistant to future pest and disease attacks. Example inoculating plants with specific strains of mycorrhizal fungi can enhance nutrient uptake, improve soil health and stimulate plant immune responses. This pre-emptive approach helps plants better withstand and recover from biotic stresses, reducing the overall impact on crop productivity.

Sustainable agricultural practices

The integration of BCAs into agricultural practices supports sustainable farming by reducing reliance on chemical inputs and minimizing environmental impact. BCAs are naturally occurring and biodegradable leading to lower risks of soil and water contamination. Moreover, their use helps in preserving beneficial organisms, enhancing soil heal and promoting biodiversity. This aligns with the principles of integrated pest management and organic farming which prioritize ecological balance and long-term agricultural sustainability.

Encapsulated BCAs for alleviation of plant biotic stressors

Encapsulated biological control agents represent a cutting-edge approach for alleviating plant biotic stressors including pests, diseases and other harmful

organisms that negatively affect crop health and productivity. By incorporating BCAs into nano-encapsulation technologies their efficacy, stability and targeted delivery are significantly enhanced providing a more effective and sustainable solution to managing plant biotic stressors [13].

Enhanced stability and longevity

One of the primary advantages of encapsulating BCAs is the enhancement of their stability and longevity. BCAs are often sensitive to environmental conditions such as temperature, humidity and UV radiation, which can lead to degradation and reduced effectiveness. Nano-encapsulation creates a protective barrier around BCAs, shielding them from these external stresses. This protection extends the shelf life of BCAs and ensures their viability over longer periods making them more reliable for application in diverse and challenging agricultural environments.

Controlled and Sustained Release

Encapsulation also facilitates controlled and sustained release of BCAs, which is crucial for effective pest and disease management. by designing nanocarriers with specific properties such as pH sensitivity or temperature responsiveness, encapsulated BCAs can be released gradually and precisely. This controlled release ensures that BCAs are at optimal times corresponding to pest outbreaks or disease conditions. Nano-encapsulated BCAs can be engineered to release their active agents in response to the presence of specific pests or pathogens, enhancing their targeted action and reducing the need for frequent reapplications [14].

Improved Bioavailability and Efficacy

Nano-encapsulation enhances the bioavailability of BCAs by improving their solubility and dispersibility. Encapsulated BCAs are more readily absorbed by plants and pests, increasing their effectiveness in controlling biotic stressors. This improved bioavailability ensures that BCAs can act more efficiently, delivering a higher impact on pest populations or disease pathogens. Encapsulated microbial inoculants can penetrate plant tissues more effectively where they can act directly on pathogens or enhance plant defenses [6].

Targeted delivery and precision

Targeted delivery is another significant benefit of nano-encapsulation. By using specific nanocarriers, BCAs can be directed precisely to the sites where they are needed, minimizing off-target effects and reducing potential

harm to beneficial organisms. This targeted approach ensures that BCAs are applied exactly where they can be most effective enhancing pest control measures and disease management strategies. Nano-encapsulated BCAs can be formulated to release their contents in response to specific environmental triggers or interactions with pest species, improving the precision and efficiency of control efforts [15].

Sustainable agricultural practices

The use of encapsulated BCAs aligns with sustainable agricultural practices by reducing reliance on chemical pesticides and minimizing environmental impact. Encapsulated BCAs are biodegradable and less likely to cause soil or water contamination, promoting a healthier and more balanced ecosystem. This approach supports integrated pest management and organic farming principles, which aim to enhance crop productivity while preserving environmental quality and biodiversity. Despite the promising advantages, the implementation of encapsulated BCAs faces challenges such as cost, scalability and regulatory considerations. Research is crucial to optimize encapsulation technologies improve cost effectiveness and address regulatory hurdles. Innovations in nanotechnology and materials science continue to advance the capabilities of encapsulated BCAs offering new opportunities for enhancing agricultural resilience and sustainability. This approach not only contributes to more effective pest control and disease management but also supports sustainable agricultural practices, promoting a healthier and more productive agricultural system [16].

Conclusion

In conclusion, nano-encapsulation offers a transformative approach to the use of BCAs in sustainable agriculture. By overcoming the inherent limitations of BCAs, such as instability and environmental sensitivity, nano-encapsulation enhances their efficacy, stability and targeted delivery. This technology ensures prolonged effectiveness and precise application maximizing environmental impacts. Various nanocarriers and encapsulation techniques provide tailored solutions for diverse agricultural challenges, supporting reduced reliance on chemical pesticides. The integration of nanotechnology into agriculture not only improves pest management but also promotes overall crop and ecosystem health. Enhanced bioavailability and controlled release mechanisms allow for more efficient use of BCAs reducing quantities and minimizing potential negative effects on non-target

organisms. Despite the promising advancements challenges like scalability cost effectiveness and regulatory considerations remain. By leveraging nanotechnology, farmers can achieve better pest control, increased crop yields and contribute to a healthier environment ensuring food security and ecological balance for future generations.

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CHAPTER 10

TRANSGENIC CROPS: CURRENT STATUS AND FUTURE PROSPECTUS

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Abstract
Plants modified through genetic engineering techniques are referred to as trans genic crops.Specificgenes have been inserted into the DNA of these crops to give them new characteristics or traits that are not naturally present in their species. The progress in agricultural science and technology has led to the ongoing revolution of GM crops, which show promise in addressing current and future challenges in commercial agriculture. Case studies in Indian cotton and Australian canola have demonstrated the potential of GM crops. Golden rice is a type of genetically modified rice (Oryza sativa) that produces beta-carotene, a precursor to vitamin A, through biosynthetic processes. The orange color of carrots and other plants is attributed to beta-carotene, a chemical that also gives rice its unique color. Transgene integration and real transgenics are therefore verified using a variety of molecular biology techniques, such as PCR, blotting, and the Enzyme-linked Immuno Sorbent Assay (ELISA), The GM seed market is currently experiencing a growth rate of 9.83–10%, and its capacity for stress tolerance is projected to enhance productivity and prosperity in commercial agriculture.

Keywords: GM crops, Transgenic crops, Biotech seeds, Biofortifiction, Gene altered food.

1. Introduction

Genetic modification (GM) is the area of biotechnology which concerns itself with the manipulation of the genetic material in living organisms, enabling them to perform specific functions.[1-2] The earliest concept of modification for domestication and consumption of plants dates back ~10,000 years where human ancestors practiced "selective breeding" and "artificial selection" – the Darwinian-coined terms broadly referring to selection of parent organisms

having desirable traits (eg: hardier stems) and breeding them for propagating their traits. The most dramatic alteration of plant genetics using these methods occurred through artificial selection of corn – from a weedy grass possessing tiny ears and few kernels (teosinte; earliest recorded growth: central Balsas river valley, southern Mexico 6300 years ago) to the current cultivars of edible corn and maize plants [3]. The use of similar techniques has also been reported to derive current variants of apples, broccoli and bananas different from their ancestral plant forms which are vastly desirable for human consumption [4].

A gram of genetically modified rice bearing "Golden Rice" (Oryza sativa) can have as much as 35 μg of β-carotene in it. Determining the vitamin, an equivalent of Golden Rice β-carotene is crucial in order to forecast the possible impact of this bio fortified grain on people that typically consume rice and have low vitamin A status. Provitamin A (β-carotene) is present in the yellow-colored endosperm of Golden Rice of which a crop that has undergone bioengineering process. In order to create Golden Rice, two enzymes phytoene synthase (psy) and phytoene desaturase (crtl) are inserted into the endosperm through an endosperm-specific glutelin (Gtl) promoter to create a pathway for the biosynthesis of β-carotene in the rice grains [5]. The orange color of carrots and other plants is attributed to beta-carotene, a chemical that also gives rice its unique color. The crop has generated a lot of controversy while being designed to assist address vitamin A deficiency, especially in children, in low-income nations where rice is a main meal (Figure 1).

Figure 1: Genetically modified rice

In addition to offering energy, staple foods should contain minerals in a form that is bioavailable. Therefore, golden rice could be an affordable staple diet for people who eat rice to help prevent vitamin A insufficiency [6]. To help other vitamin A-deficient populations with diverse food cultures, alternative provitamin A-containing staple foods including corn, cassava, sweet potatoes,

and sorghum should be created. GM Foods: GMOs are used in the production of GM foods. They have been created to increase crop productivity, disease and insect resistance, and climate flexibility.

Tobacco was the first crop plant to be genetically modified, with reports surfacing in 1983. It was created by combining an antibiotic-resistant gene with the Agrobacterium T1 plasmid to create a chimeric gene. The chimeric gene was injected into the tobacco plant as a result of the tobacco being infected with Agrobacterium transformed with this plasmid. Using tissue culture methods, the gene was isolated from a single tobacco cell, and a new plant was created from it [7]. In 1986, the US and France conducted the first field tests of genetically modified plants. Tobacco plants were modified to withstand herbicides [8]. The first business to genetically modify plants to withstand insects was Plant Genetic Systems, established in 1987 by Marc Van Montagu and Jeff Schell. The company manufactured tobacco with genes that produced insecticidal proteins derived from Bacillus thuringiensis (Bt) [9]. Figure 2 depicts the GM tobacco leaves.

Figure 2: Genetically modified tobacco leaves

Bt cotton is the first commercially released genetically modified plant in India. In partnership with the American corporation Monsanto, MAHYCO (Maharashtra Hybrid Seeds Company) produced this. Bt cotton, introduced in India in the early 2000s, was the first genetically modified crop to be commercialized in the country. It is designed to resist bollworm, a pest that affects cotton crops (Figure 3).

Figure 2: Genetically modified cotton

Although Bt cotton initially promised higher yields and reduced pesticide use, the impact has been mixed. While cotton production increased significantly, farmers' debt and environmental concerns have also increased.

Genetic Testing Techniques For Gm Crop Identification

When GM crops and their products are introduced to the market, they must be closely watched and the type and quantity of GM components must be known. It is therefore necessary to develop trustworthy techniques for the detection, identification, and quantification of genetically modified crop types and their products, as labeling laws and trade requirements differ throughout countries. Transgenes in GM crops can be detected at the DNA level, at the transcriptional level using the transgene's mRNA, or by using the trans protein that is produced. Numerous other techniques, including as mass spectrometry and chromatography, are also important for GMO testing. Figure 4 provides an overview of the test procedures used to identify and detect genetically modified crops. Each test procedure has importance and worth of its own.

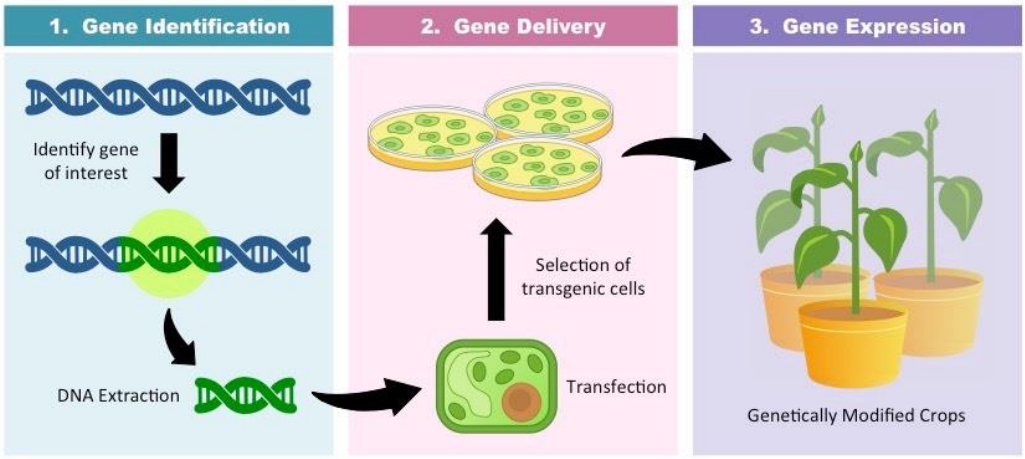

Figure 4: Methods of Genetically modified plants

The transgenic, or introduced gene, may come from distinct species within the same kingdom or from a different kingdom [10]. The genetic transformation process involves a number of phases, including the selection and identification of the target gene (transgene), isolation from the parent organism, and cloning into an appropriate plasmid carrier is now a crucial tool for agricultural improvement (Figure 5). The creation of an expression vector with all necessary regulatory components, promoters and terminators to control transgenic expression in the intended plants came next [11].

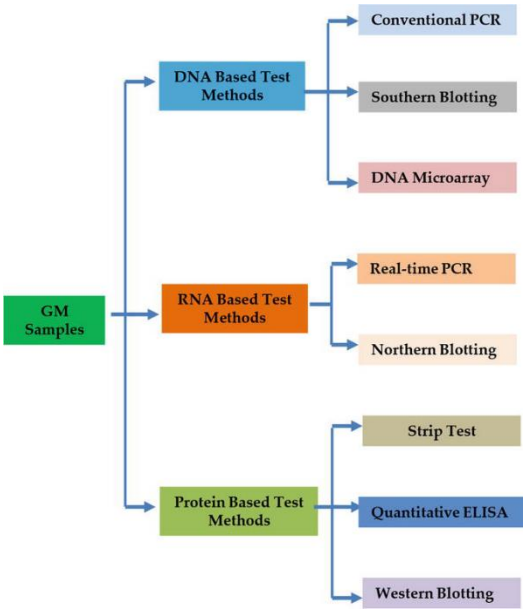

Figure5: Techniques involved in transgenic crops.

Furthermore, a second gene cassette selection is included in the expression vector, and it functions as the main selection agent for potential transgenic cells grown in artificial plant conditions. Depending on the type of work, two types of selection markers are typically used: antibiotic and visual selection markers. Prior to transformation, the final expression cassette is confirmed by a variety of molecular biology techniques and replicated in appropriate bacterial media [12]. Two methods are typically available for integrating the final expression cassette into plants: (i) direct DNA delivery system, which involves coating DNA on gold or tungsten particles and firing the mixture onto plant tissue under specific helium gas pressure; (ii) introduction of gene through biological vectors, such as the disarmed Ti-plasmid of Agrobacterium tumefaciens. Transgenes have been introduced into plants with success using both techniques. Figure 6 depicts the general process for separating genes, cloning them, transforming them, and choosing potential transgenics.

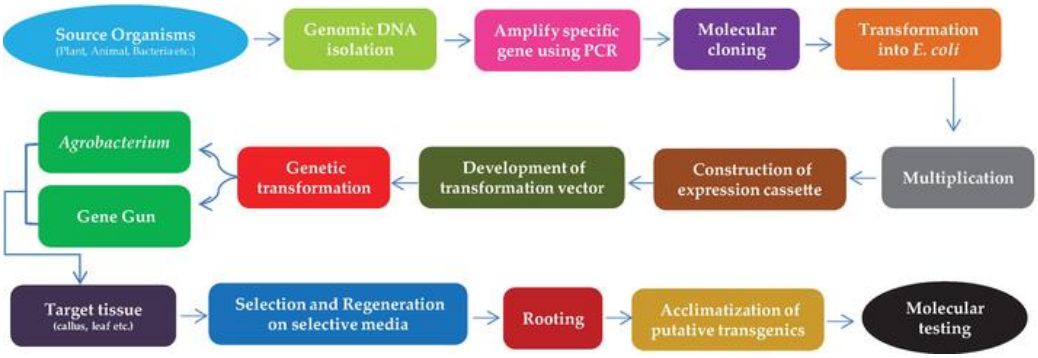

Figure 6: General process for separating genes, cloning them, transforming them, and choosing potential transgenics.

Transgenic soybean, maize, cotton, and canola were cultivated on about 90 million hectares in 2005, mainly in the USA (49.8 million ha) and Argentina (17.1 million ha), demonstrating the benefits of transgenesis for enhancing crop agronomic performance (www.isaaa.org). For this reason, substantial equivalency is the primary concept used to assess the safety of modified cereals (OECD, 1993) [13]. Several transgenes have been effectively inserted into the nuclear genomes of different plant species up to this point. Rice, soybean, maize, cotton, canola, potato, cassava, squash, papaya, groundnut, oilseeds, and a variety of fruits and vegetables are among the main crops where transgenics are commercially available [14]. Micronutrient deficiency has

skyrocketed in recent years, particularly in poorer nations. The most common deficiencies, according to the World Health Report, are those in vitamin A, iron, zinc, and iodine; these deficiencies significantly contribute to the different health disorders [15]. Chronic Health Issues Associated with Micronutrient Malnutrition Because human bodies are complex, they require both macronutrients and micronutrients for survival and optimal operation. Small amounts of micronutrients and substantial amounts of macronutrients are needed.

3. Applications Of Gmo: -

Some microorganisms are being explored as potential biodegrades and providers of clean fuels in the future. Furthermore, recombinant vaccines may one day be made from genetically engineered plants. In fact, the idea of an oral vaccine expressed in plants (fruits and vegetables) that people could directly consume is being investigated as a potential countermeasure to the spread of disease in developing nations. This approach would significantly lower the expenses related to carrying out extensive vaccination campaigns. Currently, research is being conducted to create plant-derived vaccine candidates against the Norwalk virus, enterotoxigenic Escherichia coli (ETEC), and hepatitis B virus (HBV) in potatoes and lettuce.

4. Hazards And Debates Associated With The Use Of Gmos

The effects of changing an organism's natural state by foreign gene expression are unknown, even when the genes being transferred are found naturally in other species. Such modifications can, after all, affect the organism's development rate, metabolism, and/or reaction to outside environmental stimuli. These effects affect not just the genetically modified organism (GMO) but also the natural habitat in which it is permitted to flourish. Human health risks associated with genetically modified foods include the potential for additional allergy exposure and the spread of antibiotic-resistant genes to the gut flora. In most cases, horizontal gene transfer cannot be replicated in an ideal laboratory setting without actively altering the target genome to boost susceptibility, as it happens naturally at a very low rate [16].

5. Conclusion

The area planted with genetically modified crops is expanding quickly, and major crops are receiving a large number of new genes. Before being commercially released, newly produced GMOs must undergo extensive molecular testing to ensure the safety of people, the environment, animals, and

other relevant microflora. Regulations approving genetically modified crops need thorough risk analyses in each individual situation. The identification and detection of genetically modified organisms (GMOs) is also very important for sample purity, food labeling, and commerce purposes. Therefore, for a thorough analysis, authenticity, and biosafety assessment of GM samples, it would be beneficial to combine the use of many testing methodologies.

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CHAPTER 11

MOLECULAR VARIABILITY, ISR ANALYSIS AND SCAR MARKER TO DETECT *MAGNAPORTHE GRISEA* INFECTING FINGER MILLET

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Abstract

Magnaporthe grisea, the causal agent of blast disease, poses a major threat to finger millet (*Eleusine coracana*) production, leading to significant yield losses in many parts of the world. Understanding the molecular variability among pathogen isolates is crucial for effective disease management and the development of resistant cultivars. This study focuses on assessing the genetic diversity of *M. grisea* isolates infecting finger millet using molecular markers, Induced Systemic Resistance (ISR) profiling, and Sequence Characterized Amplified Region (SCAR) markers.

A collection of *M. grisea* isolates was obtained from diverse agro-climatic regions and subjected to molecular characterization through Random Amplified Polymorphic DNA (RAPD) and SCAR marker analysis. The results revealed considerable genetic variability among the isolates, indicating the evolutionary adaptability of the pathogen. ISR analysis was conducted to evaluate the defense responses in finger millet varieties, highlighting the role of specific biochemical pathways activated upon infection.

Additionally, SCAR markers developed from RAPD-derived fragments enabled precise and reliable detection of pathogenic strains of *M. grisea*. The use of SCAR markers offers a valuable diagnostic tool for early identification and monitoring of virulent isolates, supporting breeding programs and integrated disease management strategies.

This integrated molecular approach enhances our understanding of *M. grisea* pathotypes and provides essential insights for deploying resistant cultivars and sustainable blast disease management in finger millet.

Keywords: *Magnaporthe grisea*, Finger millet (*Eleusine coracana*), Molecular variability

Introduction

Finger millet (*Eleusine coracana* (L) Gaertn.) commonly known as ragi is one of the most important millets crop and largely grown in India. It is grown successfully in areas where rainfall is about 350 mm and temperatures more than 30°C. Finger millet has an annual production of 1.89 (2009-10) million tonnes with the cultivated area of 1.26 million hectares in India. It occupies approximately 8 per cent of the area and 11 per cent of the production of all millets in the world. It is estimated that some 10 per cent of the world's 30 million tonnes of millet produced is finger millet (Dida *et al*., 2008). Perhaps 4.5 million metric tonnes of grains are produced annually on as much as five million hectares throughout the world. Almost the entire production is confined to Africa and to Asia. India alone produces between 40 and 45 per cent of the total world production and most of the rest of ragi millet is produced in Central Africa.

It is widely consumed as the staple food in the rural community of Tamil Nadu, Andhra Pradesh, Karnataka and Maharastra and the straw is also used as cattle fodder. It acts as a rich sources of calcium, carbohydrates, proteins, trace minerals, iron, niacin (a vitamin of B-complex group) and methionine (an essential amino acid). It is one of the weaning foods for babies (6-7 months onwards) and is also a good wholesome diet for diabetics. Thus, offering the user with benefits which would normally accrue to milk and its products.

Even though, finger millet is known to be one of the hardiest crops, it is affected by large number of diseases such as blast, foot rot, smut, streak and mottling virus (Govindu and Shivanandappa, 1967). Finger millet is vulnerable to leaf blast caused by *Pyricularia grisea* (Harinarayana, 1986). High seed yield loss (less than 50 per cent) is reported (Sastri, 1989). This disease occurs almost every year during rainy season but the extent of crop loss depends on the severity and the time of onset and is the most devastating disease affecting different aerial parts of the plant at all stages of its growth starting from seedling to grain formation. Yield loss due to blast may be around 28 per cent but under favorable conditions it may go higher to 80-90 per cent. Blast of ragi is caused by the fungus *Pyricularia grisea* (Cooke) Sacc. anamorph of *Magnaporthe grisea* (Hebert) Barr is a heterothallic, filamentous fungus, pathogenic to almost 40 plant species in 30 genera of Poaceae including *Eleusine* (Rossman *et al*., 1990). The fungus produced lesions on leaves, necks and discolours the grains. The leaf spots are typically elliptical. The center of the spot is usually grey or whitish and the margin is usually brown or reddish brown.

Globally, random amplified polymorphic DNA (RAPD) markers are also reported to be useful in identification (Kumar *et al*., 2010; Singh and Katoch, 2008). The structure and dynamics of the population of *P. grisea* need to be understood thoroughly if resistant genes are to be utilized ultimately to implement the strategies for management of blast. Virulence studies using host differentials are labour intensive and are further confounded by inoculation techniques and environmental conditions. In this regard, molecular methods have become alternative tools to characterize isolates of the blast pathogen (Babujee and Gnanamanickam, 2000).

The Internal Transcribed Spacer region is now the most widely sequenced DNA region in fungi. It has typically been most useful for molecular systematic at the species level and even within the species (e.g. to identify geographical races). Because of its higher degree of variation than other generic regions of rDNA (Small Sub Unit and Large Sub Unit) variation among individual rDNA repeats can sometimes be observed within both the ITS and IGS regions.

Isolation of pathogen and its pathogenicity test

Blast pathogen was isolated and cultured from the infected plant source. Pathogenicity of *M. grisea* on Ragi KM 252 was confirmed by artificial inoculation using the conidia harvested from the maize stem bits and by providing high humidity. The symptoms were developed after seven days of inoculation. Radjacommare *et al*. (2004) to prove the pathogenicity of *M .grisea* on ragi. Srinivasachary *et al*. (2002) and Priya (2008) also adopted the same method to prove the pathogenicity for the various isolates of *P. grisea* on the susceptible rice cultivar TN1.

Standardization of culture media for the growth of *M. grisea*

Five natural media were evaluated for the growth of *M. grisea*. The growth was rapid on PDA followed by Ragi leaf Agar (RLA), Ragi flour agar (RFA) and was slow on Oat meal agar and V8 juice agar. The growth was uniform in each of the natural medium. Srivastava *et al*. (2009) compared the growth of cultural characteristics of *P. grisea* isolates from finger millet on media derived from finger millets and rice *viz.* PDA, ragi leaf agar, ragi flour agar, rice leaf agar, oat meal agar. Results revealed that ragi flour media recorded better growth and sporulation of *P. grisea* at 28 °C and pH 7.5 than other medium.

Ou (1985) reported that the morphology of *P. grisea* was found to vary greatly with the medium and isolates used. In the present investigation, the mycelial

colour was white on PDA, oat meal agar, ragi leaf agar and V8 juice agar while it was blackish white on ragi flour agar. Flat growth was observed on PDA, oat meal agar and ragi flour agar while it was fluffy on ragi leaf agar and V8 juice agar. However, when all the isolates under study were on PDA, they were grey and whitish grey coloured.

Spore induction by incubating the culture

Sporulation was observed on 5 to 6 day old culture of *P. grisea* grown on two per cent rice polish agar incubated at 25-27 °C (Leaver *et al*., 1947). Isolates of *P. grisea* from finger millet, rice and pearl millet were found to sporulate on oat meal agar, rice agar, rice polish agar, PDA and malt extract agar when cultures were incubated at 27±1 °C for eight days (Kumar and Singh, 1995). Dinakar and Muralidharan (2006) observed sporulation of the fungus on PDA, oat meal agar in 7 days old cultures maintained at room temperature. In the present study, our repeated attempts to induce sporulation of *M. grisea* on the surface of the above mentioned media at 28±2 °C were unsuccessful. Hence, attempts were made to induce sporulation by incubating the culture grown on natural media in moist Petri dishes at 25±1 °C. PDA which was found to be the best for culturing the fungus also was observed to support maximum spore induction indirectly when the culture grown on the medium was incubated. However, the next one was ragi leaf agar and ragi flour agar which was found to be an alternative to PDA for culturing the fungus. Finally attempts were made to find a simple method that would hasten the sporulation of the fungus. The fungus cultured on maize stem pieces sporulated quickly when compared to ragi. Spores were also dense on maize as against ragi. Priya (2008) and Nagendran (2011) was also adopted the same method to induce the spore on maize stem bits.

Measurement of spore size

The spores of the *M. grisea* isolate Mg 11 were significantly bigger than the other isolates. Among the ragi isolates, the spores of Mg 2 isolates were smaller in length and width than that of the others. Ou (1985) states that the size of spores of the blast fungus varies among the isolates depending on environmental conditions.

Akoi (1935) measured the spore size of different isolates of *P. grisea* from rice cultured on PDA and reported that the average length ranged from 21.2 µm to 28.4µm. In our study, the spore length of ragi isolates of *M. grisea* cultured on maize stem pieces ranged from 15 µm to 23 µm. Kulkarni and Patel (1956)

observed variations in spore length and width of *P. setaria* from *Setaria italica* due to the effect of artificial media. They have also noticed that temperature had no effect on the width of the spore while length was affected. Priya, (2008) reported that spore size was varied between different isolates. However, in our study spores induced on maize stem pieces were measured for the ragi and non-ragi isolates of *M. grisea* from *Echinocloa crusgalli*.

Pathogenicity test

Pathogenicity of *M. grisea* on ragi was confirmed by artificial inoculation using conidia and providing humidity to the susceptible variety KM 252 and symptoms were developed after seven days. Srinivasachary *et al.* (2002), Priya (2008) and Nagendran (2011) to confirm the pathogenicity of *M. grisea* on ragi.

Cross-infectivity test between different hosts

Cross infectivity test was conducted to know the pathogenicity of finger millet, rice and weed. The results show that there was no cross infection between the rice and ragi, but infection was observed in weed host. Thomas (1940, 1941) found that the ragi strain of *Pyricularia* fails to infect rice and ginger but does infect wheat, barley and oats quite readily. He also found that strains from rice and *Panicum repens* would infect only its own host, and the strains from ragi and *Setaria italica* were capable of infecting wounded leaves of each other but would not infect rice or *Panicum repens.* Ramakrishnan (1948) also reports that the ragi pathogen readily infects bulrush millet, maize and *Dactloctenium aegyptium* but would not inflict rice and *Digitaria marginata.* Finger millet blast, which occurs in almost all millet growing areas, is caused by *M. grisea* isolates pathogenic to *E. coracana* but not rice (Ramakrishnan, 1948). Cross inoculation tests carried out with *M. grisea* isolates from *Eleusine* and *Oryza sativa* show that the isolates are host-specific. This result agrees with those of Kato *et al.* (1977) and Todman *et al*. (1994), who also found that *Magnaporthe* isolates from *E. coracana* failed to infect rice and *vice versa*. Kumar and Singh (1995) have reported contradictory results regarding the ability of the pathogens from rice and finger millet to cross-infect. The reasons for this variation appear to be the environmental conditions provided during experimentation in addition to the nutritional status of soil (Asuyama, 1965; Ou, 1985).

Cross infectivity between leaf, neck and finger blast pathogens

There is no organ specificity between the isolates collected from different plant parts. All the three isolates collected *viz.*, from leaf, neck, finger has the ability

to infect and produce lesions on the cross inoculated plant parts. The leaf derived isolates were able to infect neck and finger, neck derived isolates able to infect leaf and finger and vice-versa. The percentage of infection depends upon the climatic condition, stage and the crop variety. Association with leaf, neck and finger blast stages is correlated by the inoculation of leaf and panicle emergence stage (Ou and Nuque, 1963). Smita puri and Kumar (2012) found that leaf, finger and neck derived *M. grisea* isolates had the ability to cross infect all the three plant parts of *Elusine coracana* and could not be separately categorized on their pathogenicity on leaf, neck and panicles. Recently, Silva *et al*. (2009) observed low frequency of 15 rare pathotype among panicle blast isolates of rice cv. Bonaca that was not present in the leaf and which may have occurred due to change in the pathotype pattern at adult plant stage before heading and resulted into differential pathogenic behavior.

Effect of fungicides on the growth *M. grisea*

The fungicides tested were Carbendazim, Ibprobenphos, Kreoxysim methyl, Sodium hydroxide, Tricyclazole, among the fungicides used, Ibprobenphos, Tricyclazole, Sodium hydroxide completely inhibited the mycelial growth of *M. grisea* which accounted to 100 per cent inhibition over control in all the three concentrations. The fungicide carbendazim in 0.05 and 0.10 per cent inhibits the mycelial growth upto 66.66 per cent respectively and in 0.15 per cent the mycelial growth was completely inhibited. This was followed by Kreoxysim methyl inhibits the mycelial growth by 61.11 per cent in 0.05 per cent concentration and 66.66 per cent inhibition over control in 0.10 per cent, 0.15 per cent completely inhibits the growth. Azoxystrobin inhibits 55.55 per cent in 0.05 per cent and 0.10 per cent, 0.15 per cent concentration completely inhibits the mycelial growth.

The earlier reports Ramanathan *et al.* (2004) indicated that Edifenphos, Mancozeb, Tricyclazole, Carbendazim + Mancozeb and Propiconazole were the most effective at all the concentration tested and inhibited spore germination of blast pathogen of finger millet. Finger millet isolates of the blast fungus has been reported to exhibit maximum sensitivity to Carbendazim, Mancozeb and Edifenphos (Kumar and Singh, 1994). Bishi *et al.* (1985) reported that blast in finger millet was economically controlled with Bavistin. Channamma *et al*. (1979) reported that Mancozeb 0.2 per cent was superior to Hinosan in reducing blast infection. Tricyclazole was proved to be significantly superior in decreasing leaf and neck blast (Prajapati *et al*., 1999). Dubey (1997) conducted

field trials of eight fungicides for control of *Pyricularia oryzae*, Tricyclazole was proved to be most effective against leaf blast disease of rice. Minami and Ando (1994) reported that probenazole induce a resistant reaction in rice plants against infection by rice blast fungus. Gouramanis (1995) found that fungicides carbendazim, pyroquilon, thiophanate methyl and chlobenthiazone reduce the leaf blast disease of rice on the other hand tricyclazole was effective in reducing the neck blast. Edifenphos @ 0.1 per cent was effective against rice blast reported by Nagendran, (2011).

Inhibitory effect of bacterial antagonists against *M. grisea*

Inhibitory effect of bacterial antagonists against the pathogen was studied under *in vitro* condition. Among the *Bacillus* isolates used, EPCO 5 had recorded the best inhibition over *M. grisea*. Sessitsch *et al.* (2004) screened 35 endophytic isolates, out of which seven isolates showed the antagonistic activity against bacterial pathogens *viz.*, *Erwinia carotovora*, *Streptomyces scabies* and *Xanthomonas campestris*. Endophytic bacterial strain, EPCO 16 from cotton plants effectively inhibited the mycelial growth of *R. solani in vitro* (Rajendran, 2003).

Bacillus species have special characteristics that make them good candidates as biological control agents. *Bacillus amyloliquefaciens* isolates produced surfactin, iturin, bacillomycine and azalomycin F, while *B. subtilis* isolates mostly synthesize surfactin and arthrobactin. Also surfactin, amphomycin, arthrobactin and valinomycin were found in culture extracts of *B. pumilus* isolates. The antagonistic activity found for the metabolites of *Bacillus* spp. associated with the synergistic effect caused by the combination of antibiotics (Asaka and Shoda, 1996). Nagendran, (2011) reported that endophytic *Bacillus* isolates *viz.*, FZB24, EPB 13, EPCO 95, EPB 8, EPB 11, EPCO 16, EPCO 26 and EPCO 96 were found to show more than 45 per cent inhibition over control against rice blast pathogen *Pyricularia grisea in vitro*.

Assay of defense-related enzymes and compounds

Peroxidase (PO)

Peroxidase is related to resistance responses including lignifications and suberization, cross-linking of cell wall proteins, generation of ROS, phytoalexin synthesis and possess antifungal activity (Sasaki *et al*., 2004). Increased PO activity has been shown in a number of resistant interactions involving plant pathogenic fungi, bacteria and viruses (Nandakumar *et al*., 2001).

Peroxidase is a key enzyme in the biosynthesis of lignin (Bruce and West, 1989). Increased activity of peroxidases has been elicited by fluorescent pseudomonads in plants such as rice (Saveetha, 2008), black gram (Karthikeyan *et al.*, 2003), groundnut (Meena *et al*., 2000), sugarcane (Viswanathan and Samiyappan, 2001), chillies (Bharathi *et al*., 2004) and cucumber (Chen *et al*., 2000). Peroxidases have been implicated in a number of physiological and biochemical functions that may contribute to resistance including exudation of hydroxy cinnamyl alcohol into free radical intermediates (Gross, 1980), phenol oxidation (Schmidt and Feucht, 1980), polysaccharide cross linking (Fry, 1986), cross linking of extensin monomers (Everdeen *et al*., 1988) and lignification (Walter, 1992) and are also associated with deposition of phenolic compounds into plant cell walls during resistance interactions (Graham and Graham, 1991). Bozso *et al*. (2002) cloned the peroxidases associated with generalized defense reactions of tobacco plants against bacterial pathogens.

Ragi plants treated with the bioformulation containing *Bacillus subtilis* (EPCO 5) with the treatment combination of seed treatment @ 10g/ kg + foliar spray @ 0.2 per cent with *Bacillus subtilis* in the variety CO 14 + pathogen + *Bacillus* showed higher induction of peroxidases when compared to other varieties PR 202 + pathogen + *Bacillus* and KM 252 + pathogen + *Bacillus* (Fig. 1). Induction of more isoforms was also noticed in ragi plants treated with the same treatment combination (seed treatment @ 10g/ kg + foliar spray @ 0.2 per cent) with bioformulations of *B. subtilis* (EPCO 5). Similarly Saravanakumar *et al*. (2009) reported that enhanced induction of PO is seen in rice plants treated with combination of *P. fluorescens* stains Pf1 + TDK1 + PY15 upon challenge inoculation with sheath rot pathogen compared to untreated plants. Activity was found to be maximum on 5th day after that it has declined. In those plants disease incidence was found to be less under glass conditions. The enhanced induction of peroxidases in endophyte treated plants might have been part of ISR which eventually reduced the pathogen infection caused by *P. grisea, R. solani* and *X. oryzae* pv. *Oryzae* (Nagendran, 2011).

Polyphenol oxidase (PPO)

Polyphenol oxidases (PPO) are enzymes which use molecular oxygen to catalyze the oxidation of monophenolic and ortho diphenolic compounds. PPO is a copper containing enzyme that oxidises phenolics to highly toxic quinines and is involved in the terminal oxidation of diseased plant tissue and role in disease resistance. In the present study, ragi plants treated with the

bioformulation containing *Bacillus subtilis* (EPCO 5) with the treatment combination of seed treatment @ 10g/kg + foliar spray @ 0.2 per cent in the variety CO 14 + pathogen + *Bacillus* showed higher induction of polyphenol oxidase compared to other varieties and uninoculated control (Fig. 2). Induction of more isoforms was also noticed in ragi plants treated with the same treatment combination (seed treatment @ 10g/kg + foliar spray @ 0.2 per cent with bioformulations of *B. subtilis* (EPCO 5) challenged with pathogen *P. grisea* individually. Activity was found to be maximum on 5th day after challenge inoculation, later it was declined gradually. Similarly, induction of specific isoforms of PPO was observed in plants treated with *B. subtilis* (EPCO 5).

Chen *et al*. (2000) reported that PGPE induced PPO activity in cucumber root tissues upon challenge inoculation with *P. aphanidermatum*. Radjacommare *et al*. (2002) and Saveetha (2008) also reported that *P. fluorescens* (Pf1) treated rice plants showed higher activity of PPO and more isoforms of PPO upon challenge inoculation with sheath blight pathogen. An increase in PO and PPO activities in both leaves and roots of betelvine treated with *Serratia marcenscens* strain NBRI1213 (Lavania *et al*., 2006). Thus, enhanced induction of polyphenol oxidase in *Bacillus* treated plants might have been a part of ISR which eventually reduced the pathogen infection caused by *P. grisea, R. solani* and *X. oryzae* pv. *oryzae* upon artificial inoculation under glasshouse conditions. Nagendran, (2011) also reported that rice plants treated with the bioformulation containing endophytic bacteria *Bacillus subtilis* (FZB24) with the treatment combination of seed treatment @ 4g/kg + seedling dip @ 4g/l + soil application @ 500g/ha + foliar spray @ 500g/ha and challenged with the pathogens (*P. grisea, R. solani* and *X. oryzae* pv. *oryzae*) showed higher induction of polyphenol oxidase.

Phenylalanine ammonia lyase

Phenylalanine ammonia lyase (PAL) plays an important role in the biosynthesis of various defense chemicals in phenyl propanoid metabolism (Daayf *et al*., 1997). PAL activity could be induced during plant-pathogen interactions (Ramanathan *et al.*, 2000; Radjacommare, 2000; Bharathi *et al*., 2004). Karthiba (2008) and Saveetha (2008) reported that increased activity of PAL was recorded in rice plants treated with microbial bioformulations challenged with *R. solani.* The endophytic bioformulations treated cotton plants were shown increased activity of PAL after challenge inoculation with *X. axonopodis* pv. *malvacearum* (Bhuvaneswari, 2005). PAL activity was found to be higher after

two days of challenge inoculation after that activity get declined in the cotton plants treated with endophytic *Bacillus* EPCO 16 and EPCO 102 upon challenge inoculation with *X. axonopodis* pv. *malvacerum* (Rajendran *et al.*, 2006). The above findings were similar with our present study where the seed treatment @ 10g/kg + foliar spray @ 0.2 per cent and pathogen inoculated plants recorded higher level of PAL. The induction was higher up to 5^{th} day thereafter the induction declines (Fig. 3)

Superoxide dismutase (SOD)

Plants produce Active Oxygen Species (AOS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). It is one of the earliest responses of plants to attempt infection by pests and pathogens (Grant and Loake, 2000). Scavengers of active oxygen species like catalase (which catalyzes the decomposition of H_2O_2) (Scandalios, 1994), superoxide dismutase (which scavenges O_2^-) (Bowler *et al.*, 1992) and peroxidase (which scavenges H_2O_2) suppress the oxidative burst (Vera-Estrell *et al.*, 1993) and inhibit tissue necrotization. Catalase and peroxidase are of particular interest because of their role in binding salicylic acid (SA), which plays an important role in induced resistance (Anderson *et al.*, 1998). Karthiba, (2008) reported that SOD activity was high in the rice treated with microbial mixture bioformulation than the untreated control. Nagendran, (2011) reported that SOD and catalase activities were more in rice plants treated with endophytic bacteria *B. subtilis* (FZB24) applied combinely as seed treatment, seedling dip, soil application and foliar application than in untreated plants and individual mode of application after challenge inoculating with pathogens *viz*., *P. grisea, R. solani* and *X. oryzae* pv. *oryzae*. In our study, SOD activity was more in the ragi plants treated with bacillus bioformulation and induction of more isoforms was also noticed in ragi plants (Fig. 4)

Similarly, cucumber plants treated with *B. pumilus* strain SE49 resulted in a rapid lignification, in response to ingress of *C. orbiculare* and total peroxidase and superoxide dismutase (SOD) activities were increased to a greater extent than those in the control treatment (Jetiyanon *et al*., 1997). Jung *et al*. (2011) reported the changes of SOD activity were apparent by 72h after bacteriocin (thuricin17 and bacthuricin F4), purified antibiotic from *Bacillus* strains treatment of soybean leaves. Saveetha (2008) also reported the increased activity of SOD and catalyse in rice plants treated with fluorescet pseudomonads upon challenge inoculation with sheath blight pathogen.

From the above evidences, it is well known that defense enzymes are induced in ragi by the application of bioformulation containing endophytic *Bacillus subtilis* (EPCO 5) against *M. grisea.* Application of endophytes through different methods showed more induction of defense related proteins, which prevented the disease both under greenhouse and field conditions.

Chitinase

Synthesis and accumulation of PR proteins have been reported to play an important role in plant defense mechanisms. Chitinases and □-1,3-glucanases have been reported to associate with resistance in plants against pests and diseases (Van Loon, 1997). In tobacco, induction of two PR proteins *viz*., □-1,3-glucanase and chitinase was noticed due to application of *P. fluorescens* isolate CHAO in response to infection by *Tobacco necrosis virus* (TNV) (Maurhofer *et al*., 1994). The studies of Kramer and Muthukrishnan (1997); Saravanakumar (2006) also indicated the similar type of role in biocontrol of pests and plant pathogens. Chitinases and □-1,3-glucanases (which are classified under PR-3 and PR-2 groups of PR proteins respectively) have been reported to associate with resistance in plants against pests and diseases (Jayaraj *et al*., 2004).

In the present study, treating ragi seedlings with endophytic bioformulation with *B. subtilis* (EPCO 5) has increased considerable levels of chitinase (Fig. 5). It attained maximum on 5^{th} day of challenge inoculation later it starts declining. Chitinase in the cultivars, with and without challenge by *M. grisea*, revealed changes in the isoform pattern by western blot analysis. It showed a single band at 85 kDa after SDS-PAGE. Western blot analysis using barley chitinase antiserum confirmed an 85 kDa chitinase. The chitinase had anti-fungal activity against *M. grisea in vitro*. These results were supported by Radjacommare *et al*. (2004).Jayaraj *et al.* (2004) reported the induction of two □-1,3-glucanases (30 kDa and 33 kDa) in rice plants against sheath blight by foliar application of B. subtilis AUBS1. In addition, western blot analysis revealed the extra induction of two chitinases with apparent molecular masses of 30 kDa and 35 kDa in rice plants compared to untreated control. In pea, seed treatment with *P. fluorescens* strain 63-28 has produced hydrolytic enzymes such as chitinases and □-1,3 glucanases. These host lytic enzymes accumulate at the site of penetration of the fungus, *F. oxysporum* f.sp. *pisi* resulting in the degradation of fungal cell wall (Benhamou *et al*., 1996). Inoculation of tomato plants with the same strain (*P. fluorescens* strain 63-28) has similarly induced the production of plant chitinases when challenged with the wilt pathogen, *F. oxysporum* f.sp. *radicis*-

lycopersici (M'Piga *et al*., 1997). ISR has been correlated with a two-fold increase in activity of pathogenesis related peroxidase and chitinase proteins. Two peroxidase and one chitinase (35 kDa) isoforms have been induced in the PGPR treated plant inoculated with the rice sheath blight pathogen, *Rhizoctonia solani* (Nandakumar, 1998). Similarly, in sugarcane, PGPR mediated ISR against *C. falcatum*, enhanced levels of chitinase and peroxidise were noticed and specific induction of two new chitinase isoforms were found when inoculated with *C. falcatum* (Viswanathan and Samiyappan, 1999). Also the activity of β-1,3-glucanase was maximum in bacterized ragi plants with Pf1 challenged with blast pathogen (*P. grisea*) and higher activity was observed at 7 days after challenge inoculation (Radjacommare *et al*., 2004). Thus, enhanced induction of chitinase and □-1,3-glucanase in endophyte treated plants might have been a part of ISR which eventually reduced the pathogen infection caused by *P. grisea*, *R. solani* and *X. oryzae* pv. *oryzae* upon artificial inoculation under glasshouse conditions.

Molecular diversity using RAPD and SCAR

The molecular variability among 14 isolates of *M. grisea* was analyzed by means of RAPD using 20 random primers. Analysis of the genetic coefficient matrix derived from the scores of RAPD profile showed that minimum and maximum per cent similarities among the *M. grisea* isolates were in the range of 78 to 87 per cent respectively. Cluster analysis using Unweighted Pair Group method with arithmetic average (UPGMA) clearly separated the isolates into two main clusters (I and II). In the present study, among the 14 isolates *viz.*, Mg13 was placed under one group I and Mg1, Mg2, Mg3, Mg4, Mg5, Mg6, Mg7, Mg8, Mg9, Mg10, Mg11, Mg12 and Mg14 were placed under group II confirming high genetic diversity among the *M. grisea* isolates. Overall, all the isolates significantly differed from each other. Our results showed maximum similarity of 87 per cent between all the fourteen *M. grisea* isolates.

In order to develop a Species-specific SCAR marker for the detection of *M. grisea* from ragi, the unique band (~478 bp) specific to *M. grisea* isolate amplified by RAPD primer OPF-08 was cloned in pGEM-T Easy vector and sequenced. Based on the sequence of the PCR product, oligonucleotides (Mg-SCAR- FP 5'AGCTACTTGGCGATGCCAG 3' and Mg-SCAR-RP 5' AGGTGACACTATAGAATAC 3') that amplify a 478-bp SCAR marker were designed. The specificity and sensitivity of the SCAR marker were also tested. The results indicated that a 460-bp fragment was amplified from DNA of all the isolates

of M. grisea tested. However, no amplification was observed in DNA extracted from other species. viz., Pyricularia oryzea, C. gloeosporioides, C. falcatum, C. capcisi, *Rhizoctonia solani.*

Genetic diversity in fungal population may be of major importance in developing a suitable method to identify the pathogen at the early stage of infection through rapid and cheaper techniques. The analysis of genomic DNA using PCR-based methods has proven to be a fast, sensitive and reliable method for determining genetic relationships among strains of the same phytopathogenic organisms (Ma and Michailides, 2007). RAPD has the potential to detect polymorphism throughout the entire genome as compared to other PCR-based techniques. RAPD markers are often used to detect genetic similarities among pathogenic strains and can also be used for designing Sequence Characterized Amplified Region (SCAR) markers for detecting the pathogen in infected plant tissues. Ladhalakshmi *et al*. (2009) used RAPD technique and developed SCAR markers for molecular identification of isolates of *Peronosclerospora sorghi* causing downy mildew disease of maize. PCR based SCAR markers are widely used for *in planta* detection of several plant pathogens (Nicholson *et al*., 1998; Larsen *et al*., 2002).

Integrated Disease Management

It was found that *B. subtilis* (EPCO 5) has performed well in treatment combination *viz*., the combination of , Seed treatment @ 10g/ kg with *Bacillus subtilis* (EPCO 5) + FS @ 0.2 per cent with *Bacillus subtilis*.This treatment recorded more plant height with more number of tillers, fingers and number of grains compared to untreated control plants. The plants recorded a significantly lower disease incidence of blast compared to untreated control plants. In support of our results, *B. subtilis* (FZB24) has performed well in treatment combination *viz*., seed treatment @ 4g/kg + seedling dip @4g/l + soil application @500g/ha + foliar application @500g/ ha on 30 days after transplanting under glasshouse conditions. This treatment recorded more plant height with more number of tillers compared to untreated control plants (Nagendran, 2011). Seed bacterization and soil application of *B. subtilis* CA32r alone did not protect chilli plants, but the combined treatments of root bacterization and soil application, the population size was maintained at a higher level compared to the other treatments which resulted in a significantly higher plant protection indicating an additive effect of SA of CA32r (Abeysinghe, 2009). Szczech and Shoda (2006) reported that seed coating of *B. subtilis* RB14-C was not effective at controlling *Rhizoctonia* damping-off on tomato but

RB14-C mixed with soil significantly controlled *Rhizoctonia solani*. Fluorescent pseudomonad strains that showed enhanced plant growth, production of catalase and lytic enzymes and exhibited higher antagonistic activity against *R. solani* were selected and used against pest and diseases in rice under glass house and field conditions both in individual as well as in combinations. Bioformulation containing *Pseudomonas fluorescens* strains Pf-1, TDK-1 and KH-1 effectively reduced the incidence of leaf folder and sheath blight disease under glasshouse and field conditions (Saveetha, 2008).

Salahaddin *et al.* (2010) has found that *B. subtilis* B49 found to be the most effective in inhibiting the growth of *Xanthomonas axonopodis* pv.*malvacearum in vitro* among 93 isolates of rhizobacteria and also found to effectively control the bacterial blight of cotton both under greenhouse and field conditions.

Even though the fungicide Iprobenphos recorded lesser disease incidence compared to the bioformulation considering the residual effect, population of natural enemies and environmental safety. Overall, application of *Bacillus* bioformulation in combination with different methods of application gives the protection against blast disease throughout the crop stand as the different methods of application provide sufficient load of *B. subtilis* (EPCO 5) than the untreated control plants under field conditions. The mechanism involved in disease resistance may endorse the action of Induced Systemic Resistance (ISR) or Systemic Acquired Resistance (SAR) or combination of both.

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CHAPTER 12

ENDOPHYTES AND THEIR ROLE IN DISEASE MANAGEMENT

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Abstract
Crop diseases occur in agricultural and horticultural crops that result in yield loss or total crop losses and leads to insecure food production. Plant disease management is achieved through the application of fungicides, antibiotics and insecticides. Frequent usage of agrochemicals and application of chemical fertilizers impart negative effects on the ecosystem and human beings. Additional impact of environmental pollution, pathogen resistance, and ecological imbalance also occur. Use of endophytic biocontrol agents, such as beneficial microorganisms and their products and metabolites safeguard the environment through the action of natural mechanism. Endophytes, include fungi, bacteria, and actinomycetes, exist in different parts and intercellular spaces of plants without causing any damages and established a mutually beneficial relationship with host plants. Plant-promoting endophytes are categorized based on their dwelling as stem (laimosphere), fruits (carposphere), leaves (Phyllosphere), seeds (spermosphere), and flowers (anthosphere). Phyllosphere (leaves) and laimosphere (stems) are well documented. Endophytes obtained their nutrients from plants and maintaining the health of plants through their metabolites and suppress the pathogen by various activities *viz*., plant growth promotion, metabolite production, induced resistance.

Key words: Endophytes, plant growth, metabolites, induced resistance

Introduction

Any organism that grows within plant tissues", was defined as an endophyte (De Bary, 1866) It is differed from an epiphyte dwelling on the surface of plants [1]. Carroll (1986) provided a new definition for endophytes as organisms that inhabit the aerial parts and living tissues of plants without causing visible infection or diseases, emphasizing the mutualistic relationship

between endophytes and plants; pathogenic and mycorrhizal fungi were excluded [2]. Carroll's definition was modified by Petrini (1991) as all organisms that colonize within plant tissues for some part of their lifecycle and do not cause symptomatic infections to the host plants, from which latent pathogens are also known as endophytes [3]. The concept of endophyte is always controversial. The definition from Petrini is commonly used in most endophyte studies. The fungi belong to *Ascomycota*, *Zygomycota*, and *Basidiomycota* are the major members of endophytic . Endophytic fungi are well known to produce various bioactive compounds. Endophytic bacteria such as *Bacillus*, *Agrobacterium*, *Brevibacterium*, *Pseudomonas*, etc. belong to a diverse group of species, ranging from gram-positive to gram-negative bacteria [4]. Wide variety of endophytes inhabited wild rice such as *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* [5]. The diversity of endophytic bacteria in plants is affected by the host and environment related factors, e.g., plant growth stages, geographical location, and climatic conditions [6]. Mangrove plants and medicinal plants in tropical rain forest harboured endophytic actinomycetes in a wide variety of plants [7]. Population of endophytic actinomycetes in plant roots are more than those in other parts of plants. *Streptomyces* and *Micromonospora* are the dominant genera and augmented as valuable resources for antibiotics and other bioactive metabolites.

Endophytes

Endophytes have more positive and direct impacts on plants because of their special ecological niches when compared to soil microorganisms. Plant growth promotion, stress mitigation, and disease resistance in host plants and competition with pathogens for space and nutrition provided good evidence for endophytes role [8, 9, 10, 11]. *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Streptomyces*, etc., are used as microbial formulations against various phytopathogens [12].

Endophytes produces variety of metabolites such as alkaloids, polypeptides, polyketides, terpenoids, etc., with significant biological activities, especially in agriculture [13,14,15]. These metabolites serve as antibiotics and insecticides [16, 17]. Bioactive metabolites helps the host plants directly or indirectly by resist biotic and abiotic stresses. Some antimicrobial compounds produced by endophytes strongly inhibiting pathogens, by decompose the cell wall of pathogens through hydrolases secretion and phytohormones released by endophytes play a vital role in plant growth [18, 19]. Certain microorganisms called plant growth-promoting microbes (PGPM) perform this function through metabolites production such as antimicrobials and volatile organic compounds

[20]. Application of metabolites from endophytes in eliciting plant resistance are still limited and need to be explored and enriched in future research.

Role of endophytes as biocontrol agents and their metabolites against diseases have been reported in the literature. The mechanisms of endophytes includes (1) competing with pathogens for niche and nutrition, (2) producing antimicrobial compounds, (3) secreting lytic enzymes, (4) inducing systemic resistance in host plants, and (5) producing plant hormones and plant growth-promoting regulators.

Endophytes and their role in biotic stress management

Biotic factors affect the physiological and molecular functioning of plants and also led to the decrease in crop productivity. Bacterial and fungal endophytes are the solution to overcome the tasks faced with conventional farming which are environment friendly microbial commodities that colonize in plant tissues without causing any deleterious effects. Endophytes enhance the uptake of nutrients such as uptake of K ions in plant tissues, nitrogen, magnesium, zinc, sulphur, and phosphorus from soil and provide to the host plant for better growth and survival, synthesis of phytohormones like IAA, ACC deaminase, and reduce the injury incited by pathogens *via* antibiosis, production of lytic enzymes, secondary metabolites, and hormone activation and decreased ethylene level are an alternate mechanism to alleviate stress conditions in various plants . Endophytes improving crops and soil health by acting as biofertilizers and biocontrol agent and developed an eco-friendly substitute to destructive chemicals for plant development and also in mitigation of biotic stress [21,22, 23]. Diversified fungal species especially entomopathogenic fungi exert long-term preventive measure for insect population [24].Various bacteria such as *Bacillus*, *Pseudomonas*, *Pedobacter*, and *Acidobacterium* involved in mineral solubilization, metabolite production, and N_2 fixation. Several fungal strains including *Beauveria bassiana*, *B. metarhizium*, *M. robertsii*, *Chaetomium globosum*, and *Acremonium* spp. are successful in plant protection [25]. Endophytic fungus has wide host range hence become advantageous as compared to other biocontrol agents. *Trichoderma viride* isolated from *Spilanthes paniculata* showed broad range activity against *Colletotrichum capsici*, *Fusarium solani*, and *Pythium aphanidermatum* [26]. Biotechnological approach *viz.*, genetic engineering and other chemical and physical methods have been used to get stress tolerant cultivars. Since it provides stress tolerance capacity for a very long time, and also, they are not ecofriendly. Thus, utilizing the potential of beneficial

endophytes present in the nature for disease management is an alternative strategy for improving plant resistance and resilience in crop varieties [27].

Distribution of endophytic microbes

Root endophytic microbes

Roots of crop plants are the main habitat and colonization for the bacterial and fungal endophytes. Entry points for bacterial colonization are root hairs, root cracks, or wounds formed by microbial or nematode activities. Other major sites for root colonization include intercellular spaces in cortex and epidermis [28]. Endophytes such as *Pseudomonas putida* and *P. fluorescens* colonized the olive through root hairs [29]. Inoculation of fungal root endophyte *Piriformospora indica* promotes plant growth, early flowering, higher seed yield, and adaptation to stresses in various host plants such as *Phaseolus vulgaris*, *Triticum aestivum*, and *Cicer arientum* [30, 31].

Phyllosphere endophytic microbes

Plant-promoting endophytes are categorized based on their dwelling as stem (laimosphere), fruits (carposphere), leaves (Phyllosphere), seeds (spermosphere), and flowers (anthosphere). Phyllosphere (leaves) and laimosphere (stems) are well documented. Phyllosphere microbes are an important component of microbial communities that live asymptomatically within leaves and also known for plant health maintenance [32]. Eventhough largest microbial habitat on Earth, (10^{62} cells) the functional roles of phyllosphere residents are still less understood. The most abundant genus of phyllosphere region is *Pseudomonas* in tomato plants [33]. Leaf endophytes including bacteria and fungi and most of the times possess five phyla, *Proteobacteria* (90%), *Actinobacteria* (2.5%), *Plancomycetes* (1.4%), *Verrucomicrobia*, and *Acidobacteria* (1.1 and 0.5%) [34]. They live inside the leaf and maintain symbiotic relationship with the host plants.

Endophytes enter leaves and stems through natural openings such as stomata and hydathodes through dispersion with the help of rain, soil, or pollinators [35]. For example, *Gluconobacter diazotrophicus* enters through stomata in sugarcane plants [36]. After reaching this site, endophyte strains multiply and form a thin layer of biofilm. Apart from this, some may enter to the inner tissues and start residing as endophytes where further microbes could colonize themselves into xylem.

Similar to bacteria, strains fungi also equally promote plant growth through nutrient recycling, i.e., carbon and nitrogen, provide resistance to pathogens and helps in leaf litter decomposition [37]. Various fungal species such as *Penicillium aurantiogriseum*, *Fusarium incarnatum*, *Trichoderma harzianum*, and *Fusarium proliferatum* have been reported from wheat plant [38]. Seed-borne endophytic microbes produce phytohormones, enzymes, and antimicrobial compounds and improve plant development through their vertical transmission. Such microbes are naturally useful for community gathering in the new seedling [39]. Seed-borne endophytes (bacterial and fungal) benefit seeds by facilitating the germination of seeds in soil.

Functions of endophytes as biocontrol agents

Bacterial and fungal endophytes involved in plant disease management was reported by researchers.Bacillomycin D protein produced by *Bacillus amyloliquefaciens* showed antagonistic activity against *Fusarium graminearum* [40] *Serendipita indica* conferred resistance against *Fusarium* and *Rhizoctonia solani* and expressed antioxidant capacity under *in vitro* condition [41]. Colonization of *B. bassiana* 11-98 in tomato and cotton seeds protect seedlings against *Rhizoctonia solani* and *Pythium myriotylum*. Mechanisms of endophytes were coiling of hyphae, induction of resistance, and production of lytic enzymes such as cellulose, 1,3- glucanases, amylase, and glutaminase, that protect the older plants from root rot and reduce disease severity in many plants. However, biocontrol nature of endophytes may be achieved through direct inhibition of pathogens or indirectly by establishing the systemic resistance in plants [42]. The other involved mechanisms include competition for space and nutrients, production of cell wall degrading enzymes, initiation of induced systemic resistance (ISR), and quenching the quorum sensing of pathogens [43, 44]. Endophytes are more protected from external factors such as radiations, temperature, and pressure when compared to epiphytes [45].

Hyperparasitism: Parasitic nature of endophytes

Hyperparasitism is frequently observed in fungi, but rarely seen in bacteria. It is one of the biocontrol strategies in which the parasitic host is plant pathogen. Instead of using chemicals, it is frequently used to protect plants against pathogens. *Trichoderma* species is the most prevalent necrotrophic hyperparasite (mycoparasite) that targets host mycelium [46, 47]. Predatory bacterium such as *Bdellovibrio bacteriovorus* use the bacterial cytoplasm as

nutrients [48]. Plant pathogenic microbes such as *Xanthomonas vesicatoria* , *Erwinia carotovora*, *Pseudomonas syringae*, are predated by *E. herbicola* [49]. *Trichoderma* spp. parasitize *Rhizoctonia solani* hyphae, inhibiting the disease production in soybean seedlings (damping off) and sugar beet (root rot) [50].

Competition for space, nutrients and infection

Pathogen adapts to nutrient-rich places such as the rhizosphere, phyllosphere, phloem, and xylem based on their survival needs. Some pathogens enter through stomata such as *Pseudomonas syringae,* while others use nectarthodes such as *Erwinia amylovora,* which causes apple fire blight disease [51, 52]. Non- pathogenic endophytes already residing in the tissue prevented the pathogens invading plant tissues that consume nutrients from host tissues. Endophytes are ubiquitous through colonization and can resist the pathogen attack through competing for resources available to pathogens. [53]. Endophytes exhibiting extensive niche overlap against Dutch elm disease pathogen. Endophytes showed high competition with respect to the utilization of sugar alcohols, monosaccharides, and tri- and tetra-saccharide [24].

Biosynthesis of siderophores

Various microorganisms including endophytes produces siderophores which are low molecular weight compounds to scavenge iron and make it available to plants. Endophytes synthesize different types of siderophore such as hydroxamate, carboxylate, and phenolate that confers plant protection against phytopathogens and enhanced the plant growth and yield by providing iron to plants under iron deficient conditions [54]. *Azotobacter, Bacillus, Enterobacter, Arthrobacter, Nocardia* and *Streptomyces* are bacterial endophytes that contain property of iron chelation [55] .

Lytic enzymes vs Plant disease antagonist

Potential antagonistic microbes exhibit biocontrol activity against pathogenic microbes through extracellular enzymes that increasingly explored as target. Numerous endophytes produce different lytic enzymes such as chitinase, cellulose, proteases, hemicelluloses, and amylase, which helps the hydrolysis of polymers [56 , 57]. Colonization of endophytes in the host cells through formation of polysaccharide and protein biofilms is achieved through

lytic enzymes [58]. Endophytes also helps in controlling plant pathogens through cell wall degradation process [59] Polysaccharides mostly comprises the component of fungal cell wall that provide structural stiffness to the cell wall of phytopathogens. Therefore, the interference in the glycosidic bonds through enzymatic lysis can deteriorate the cell wall and thereby cause cell death.

Induced resistance vs plant infection

Induction of resistance in plants is an indirect mechanism through which endophytes inhibit pathogens. Endophytes possess the property of decrease disease susceptibility on pathogen attack by triggering induced resistance in their host plant [60]. Phytohormones such as ethylene or jasmonic acid mediated resistance patterns in plants and systemic acquired resistance (SAR) linked with the salicylic acid regulation is the known signalling pathways. Root colonization by endophytes and expression of pathogenesis-related genes is often correlated with the elicitation of induced systemic resistance against infection. For example, root endophytic *Fusarium solani* shown to reduce infection in tomato by activating pathogenesis-related genes such as PR5 and PR7 [61]. The endophyte *Bacillus pumilus* along with synthetic benzothiadiazole triggered ISR in contrast to bacterial spot disease in pepper occurred due to *Xanthomonas axonopodis* [62] Powdery mildew pathogen *Blumeria graminis* was supressed by *Epichloe* spp. that showed the ability to expression of salicylic acid defense mechanism [63]. Expression of pathogenesis-related PR1 protein and callose deposition by *Bacillus cereus* induced ISR against *Botrytis cinera* and simultaneously activated the salicylic acid and jasmonic acid / ethylene [64]

Plant growth-promoting mechanisms in endophytes

Endophytes encourage the plant growth directly/indirectly and impart beneficial effects on their host plant. Plant growth occurs directly through endophyte–pathogen interaction by the regulating nutrients *viz*., phosphorous and nitrogen, modulating level of hormones. As Indirect means enhanced plant defense, endophytes causes biocontrol of phytopathogens through the production of antibiotics, regulation of defense mechanism by induced systemic resistance, reduce the quantity of iron assimilation to pathogen, and pathogen inhibition via volatile compounds . Direct mechanisms involved in plant development is discussed.

Phytohormones production

Endophytes secrete gibberellic acid, cytokinin, auxins such as indole acetic acid, and ethylene. These hormones stimulate plant growth by regulating structural and morphological changes such as increase the root biomass and root surface area and control the rate of vegetative growth [65] Certain endophytic strains of *Azospirillum brasilense* produced other indole-related compounds such as indole-3-lactic acid (ILA) and indole acetamide (IAM) as an intermediate during the auxin biosynthetic pathways.

Endophytic production of antifungal and antibacterial secondary metabolites

Metabolites secreted by endophytes directly or indirectly enhance the tolerance of the host plant to various stresses and making them beneficial to the plants by improving their potential to serve as promising biological agents for plant disease control. For example, *Pyricularia oryzae* Cav., a rice blast causing fungus was efficiently controlled by the application of endophytic microbes [66]. The fungal endophytes of *Populus alba* enhanced the host's tolerance to the pathogen *Venturia tremulae*, as described by. [67].

Most of the secondary metabolites produced by endophytes exhibiting good antibacterial and antifungal activities and preventing the growth of harmful microorganisms. Alkaloids, phenols, flavonoids, peptides, steroids, and terpenoids are isolated from bacterial and fungal endophytic strains constitute the metabolites. Several endophytes produced lipopeptides and showed antimicrobial and surfactant activities and also well known for their antibiotic activity. *Bacillus amyloliquefaciens* strain produces lipopeptides having biocontrol activity against *Erysiphe cichoracearum* (fungal pathogen). *Bacillus* sp. produced fengycin, iturin, and surfactin helps to inhibit the growth of fungal pathogen [68].

Bio control mechanism through quorum quenching

Growth and metabolism in single-cell microorganisms such as bacteria is controlled by quorum sensing (QS) signalling mechanism. Most of the traits are managed by density-dependent cell-to-cell communication in endophytes by quorum sensing as well a key controller of virulence in pathogens [69]. Biofilm formation, toxin production, antibiotic resistance, exopolysaccharides (EPS), and degradative exoenzymes secretions are highly regulated by quorum sensing signalling these factors are responsible for virulence. This mechanism takes

place through small diffusible signalling molecules called autoinducers [70]. The pathogenic bacteria such as *Pseudomonas* and *Ralstonia* primarily use acylated homoserine lactones (AHLs) to communicate while producing virulence and cause great damage to crops [71]. Hence, antiquorum sensing approach could be harnessed to trigger the phenotype of pathogen to block infection [72]. Virulence-associated activities such as modification of signals, catalysis of degrading enzymes such as AHL-lactonase, and inhibition of signal synthesis are regulated by quenching process [73].

Endophytic bacteria and fungi provide large number of bioactive molecules, which act as an inhibiting agents including QS quenching enzymes such as lactonase, acyclase, and QS inhibitor molecules [74]. The above agents can provide promising approach to control phytopathogens and suppress virulence expression in them. They also helps in degrading quorum-sensing signals from pathogenic microbes and disrupt intercellular communication [75]. Endophytes with quorum quenching activity make weaking of virulence factors instead of killing the microbes or limit the cell growth. Further it effectively reduces the selective pressure associated with bactericidal agents [72]. Protease activity (LasA and Las B protease activity) responsible for virulence was correlated with decrease in biofilm formation [77]. Additionally, the application of this strain as a biocontrol agent considerably reduce soft rot disease produced by *D. zeae* EC1 through the suppression of tissue maceration in numerous host plants [78]. These observations demonstrate that QQ strains have huge potential to reduce the disease harshness due to QS-modified pathogenic bacteria. Engineered endophytic bacterium through introducing quorum-quenching gene is achieved by antivirulence activity. For example, to control *Burkholderia glumae* causal organism of grain rots of rice, an *N*-acyl-homoserine lactonase (*aiiA*) gene from *Bacillus thuringiensis* was inoculated into *Burkholderia sp.* KJ006 to repress *N*-acyl-homoserine lactone [79]. Thus, quorum-quenching microbes provide great potential as biocontrol agents. Being compatible in nature endophytes occupies most of the cellular space without leaving space for later-invading phytopathogens [80].

Plant defense mechanism vs biotic stress

Fungal parasites inhibit the growth of plants by killing the host cell through toxin production or feed on living host cell as biotropic fungi. Haustoria plays a major role in absorbing nutrients from host tissues in some biotropic fungi [81]. Immune system of the plants gets activated on pathogen attack that prevents the pathogen entry and terminate their growth. Physical barriers such

as waxy cuticles, rigid cell wall, and trichomes are the primary defense to avoid phytopathogens. Cuticle not only restricts the entry of liquid and gas fluxes but also protects plants against pathogens, xenobiotics, and irradiation [82].

Defense mechanism achieved through different concentrations and compositions of compounds such as amines, peptides, alkaloids, cyanogenic glucosides, phenolics, polyacetylenes, non-protein amino acids, and quinines, contribute significantly to disease reduction in plants [83]. Production of phytoanticipins and phytoalexin as preformed and induced defense mechanisms also activated after pathogen attack. Phytoalexins are low molecular weight compounds that possess antimicrobial properties. There are wide varieties of phenolic compounds, which assist in phenotypic plasticity and act as inhibitors [84]. Mainly, hydroquinones, caffeic acid, gallic acids, hydroxycinnamates, and 5-hydroxynapthoquinones are effective allelochemicals [85]. Plant hormones such as salicylic acid, ethylene, and jasmonic acid play additional role in biotic stress signalling. The microbe-associated patterns (PAMP) such as lipopolysaccharides, peptidoglycan, and bacterial flagellin developed in plants as an innate immunity system. Such immunity is called PAMP triggered immunity. Plants are recognized through herbivore-associated molecular patterns (HAMPs) [86]. Other immune response includes transcription methods in the host nucleus and recognizing Avr proteins that are avirulent in nature. Effector triggered immunity arouses hypersensitive responses (HRs) and causes programmed cell death (PCD) in diseased and nearby cells [87]. Systemic acquired resistance (SAR), a long-lasting and broad-spectrum pathogen resistance against secondary infection is conserved in diversified plants. Salicylic acid is increased in tissues that occur systematically after localized exposure to a pathogen or after treatment with synthetic or natural compounds [88].

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CHAPTER 13

MICROBIAL INTERACTION AND SYNERGISTIC EFFECT IN DISEASE CONTROL

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ABSTRACT:
The increasing global demand for an ecofriendly alternative to a chemical pesticide and fertilizer. The interaction between the microorganism especially synergistic relationship plays a crucial role in uplifting the plant growth and protection against various challenging environmental stress both biotic and abiotic factor. The investigation is made on key microbial groups including plant growth promoting rhizobacteria, has their efficient role in disease suppression. The beneficial microorganism involved in synergistic effect where the plant growth is promoted by various mechanism that inhibit the disease-causing pathogen. In order to assess the efficiency of beneficial microbes against disease control by experimenting various In vitro screening techniques. The efficacy of beneficial microbes assessed by green house experiment. This study demonstrates the synergistic impact by microbial interaction in the effect of disease control in promoting the plant defense mechanism and a promising approach in sustainable agriculture practices.
Key words: microbial interaction, synergistic effect, disease suppression, green house experiment

INTRODUCTION:

The recent trends in using beneficial microbes for disease control that highlights the huge demand for eco- friendly alternatives to chemical pesticides and fertilizers. In general, Microbial interaction cites to the various ways that microbes interact with each other and with their environment. This interaction may be beneficial (mutualistic or synergistic), sometimes neutral or harmful(antagonistic). This beneficial interaction involved to enhance plant growth and help to fight with various environmental stress, especially synergistic interaction plays a crucial role by outcompeting or inhibiting the

growth of pathogenic micro-organism in order to control the plant disease. In the study of beneficial microbes most commonly plant growth promoting rhizobacteria(PGPR), mycorrhizal fungi and endophytes plays important role in disease control in which PGPR involved in synergistic effect where plant roots are colonized and plant growth is promoted by various mechanism of action on the other hand synergistic relationship with fungi – mycorrhizal fungi form symbiotic relationship with roots of the host plant which in nutrient absorption and providing protection against various soil-borne pathogen. However, destructive interaction with pathogenic microorganism leads to plant disease. In the mixed population of microbes there are various beneficial and deleterious microbes in rhizosphere region, in that case efficient biocontrol microbes are imperative to eliminate pathogens and to fight challenge charged by pathogens.

The objective of the study is to highlight, describe and discuss the types and various mechanism of microbial interaction and also reveals some methods and examples for the synergistic effect in disease control. This study also focuses on the microbial interaction which are essential for understanding disease development and biocontrol in plants. Disease suppression in crops can be accomplished by influencing physical, chemical and microbiological environment through various management practices.

Mechanism of action:

Inhibition of pathogen by antimicrobial compound (antibiosis):

Antibiosis is a term in which the chemical compounds secreted by antagonists inhibit or kill the potent pathogen. Some antagonists suppress pathogens by producing antibiotics and other antimicrobial metabolites. Many bacterial genera like *Bacillus, pseudomonas,* etc., are predominantly involved in the production of antibiotics and antimetabolites. In general, antagonistic microorganisms are screened by in vitro assays but sometimes they may exaggerate the importance of the metabolites due to artificial conditions when compared to the actual effectiveness of these metabolites or antibiotics in natural habitats.

Competition for iron through the production of siderophore:

Iron is crucial for the virulence of pathogens and growth that play an important role in biological control of pathogenic fungi.

To obtain ferric iron, microbial antagonists produce low molecular weight compounds (siderophore) under iron-limiting conditions.

Siderophores are the chelating compounds that facilitate the tight and stable complex by achieving binding with ferric ions and transport into the cell

Indirect interaction with pathogens: Competition

The germination, growth, and survival of plant pathogens depend upon nutrient uptake. This is due to necrotrophic pathogens which kill plant tissues and use the released nutrients to serve themselves as primary colonizers on the other hand non-pathogenic microorganisms which have a saprophytic lifestyle often colonize the same tissue, leading to competition for nutrients and space. As a result, necrotrophic pathogens, as primary colonizers have the upper hand and thus compete effectively. These pathogens undergo the saprophytic stage, in this stage, they are susceptible to competition from other microorganisms for nutrients making these competitors efficient biocontrol agents. Effective microbial biocontrol agents outcompete pathogens for essential space and nutrients thus preventing the pathogen from infecting the host plant.

Induction of plant resistance mechanism:

Induced resistance refers to the enhancement of a plant's physical or chemical defense mechanism by both biotic (living organism) or abiotic (environmental factors). Induced resistance has two important types of resistance such as:

Induced systemic resistance: triggered by beneficial microbes, induced systemic resistance uplifts the ability of plants to defend against multiple pathogens.

Systemic acquired resistance: the direct response to targeted pathogens that are involved in causing tissue damage, spreading resistance throughout the plant and also to neighboring plants.

In this, the important molecules that induce defense pathways by plant receptors are called microbe-associated molecular patterns (MAMPs). These molecules are produced in lower concentrations but are efficient in triggering rapid plant defense response.

Mycoparasitism:

Parasitism is commonly referred to the ability of a microorganism to parasitize and degrade fungal spores of plant pathogen whereas mycoparasitism refers to the ability of fungi to parasitize spores, sclerotia, hyphae, and other fungal structures. The process in mycoparasitism may consist of the host, followed by various steps such as directed growth,

contact, recognition, attachment, penetration, and exit are the key terms that prove the transfer of nutrients from host to mycoparasite. During mycoparasitism, the penetration of cell wall degradation is commonly observed.

Hyper parasitism:

The term involves interaction between two organisms in which one organism, hyperparasite, gains nutrients from a parasitic host. There are divisions such as **Biotrophic mycoparasitism** where hyperparasites gain nutrients from the living host fungus without killing it on the other hand **Necrotrophic hyperparasites** kill host cells before invading them**.**

Dual culture technique:

The dual culture method assesses the antagonistic effect of two targeted organisms against disease-causing pathogens. For example, in the case, *of pseudomonas fluorescens* (a bacterial antagonist) and *trichoderma harzanum* (a fungal antagonist) against a fungal pathogen *sclerotium rolfii*. There are certain steps to be followed to achieve a dual culture technique where the preparation of media is the main step that facilitates the growth of antagonist bacterial and fungal organisms and the cultures were inoculated in the appropriate medium. Then these inoculated plates were incubated to achieve the growth and interaction between the microorganisms. After witnessing the increase, the zone of inhibition is measured which indicates the effectiveness of the antagonistic organism in suppressing the growth of disease-causing pathogens. The results will indicate the extent to which the biocontrol agents inhibited the growth of disease-causing pathogens, highlighting the potential of these microorganisms as effective biological control agents in agricultural practices. Thus, the dual culture technique effectively demonstrates the antagonistic interactions between biocontrol agents and plant pathogens, providing insights into their potential application in sustainable disease management strategies.

Microbial interaction and synergistic effect:

By dual culture technique, the beneficial strains play a significant role in improving plant health by microbial interaction and synergistic effect. This illustrates how microbial interaction induce the defense mechanism in plants.

Enhanced Antimicrobial Activity: Beneficial microbes should exhibit significant antimicrobial activity to inhibit disease-causing pathogens. This

enhances the defensive mechanism in the host plant by disrupting pathogen development

Biocontrol Efficacy: The dual culture technique evaluated the antagonistic effect of biocontrol agents where the beneficial microbes compete or inhibit pathogens. In such interaction, it shows not only suppression of disease but also nutrient acquisition

Improved Plant Resilience: The microbial strains that confer disease resistance and enhance nutrient uptake and plant resilience against biotic stresses by synergistic interaction among microbes. This resilience is critical for sustainable agriculture, as it reduces the using chemical pesticides.

In vitro screening:

These are the some of the basic experiments that gives an overview of various In-vitro screening test that evaluate the plant growth and biocontrol ability of beneficial microbial strains.

Phosphorous and Nitrogen dissolution test:

Nitrogen and phosphorous are the most important essential elements that play a vital role in crop production by enhancing the growth and development of a plant. The phosphorous test has been carried out by incubating the potent isolate in organic and inorganic phosphorus media at 28℃ for about six days. Then results were analyzed by the growth of the colony, phosphorus solubilization and phosphate dissolution circle to assess the effectiveness. The nitrogen dissolution test, was performed by inoculating the isolate into protein media and incubated at 37℃ for about 2days. The colony growth and nitrogen solubilization zone was measured to evaluate the function of nitrogen dissolution test.

Determination of Nitrogenase Activity:

To Investigate the effect of beneficial strains that promotes the growth of plant and biological control for various environmental stress. In order to determine the nitrogenase activity the microbes were cultured in the nitrogen free media (Ashby's and nitrogen free agar plates) at 28±1℃ for 8days. The result was evaluated by observing the colony growth on the plates and estimating the ability of the beneficial strains to fix nitrogen. This experiment has a vital role in enhancing plant growth even in challenging environment.

Phosphate solubilization:

The Phosphate solubilization test evaluates the ability of microbes to convert complex insoluble phosphate forms to soluble ones that are immediately absorbed by plants which plays a major role in soil fertility and plant growth promotion. They play a crucial role by improving soil fertility and providing sustainable agricultural practices. The activity was tested and interpreted by observing the formation of a clearing zone around the colonies after incubation. This result indicates the utilization of phosphate and thus proves the ability to soluble inorganic phosphate.

Siderophores detection:

Siderophore production is one of the key terms in competition for iron and ferric complexes. They are grown in an agar medium that is particular for the selected strain and interpreted by detecting the change in color of the media around the colony as positive for siderophore detection.

Indole acetic acid production:

The ability of beneficial strains that produce Indole acetic acid (IAA) is determined by using Salkowski's calorimetric method. The beneficial strains were inoculated and grown in a broth (TYC) and incubated. The broth culture was centrifuged and the supernatant was mixed with Salkowski's reagent and recommended to keep in the dark at room temperature. The result is interpreted by observing the color change and measuring the optical density by comparing it with the already-known values of indole acetic acid (IAA)

GREEN HOUSE EXPERIMENT:

In a Greenhouse experiment, the impact of various treatments on the selected plant has been assessed and also reveals the effectiveness of various biocontrol agents including arbuscular mycorrhizal fungi, bacterial and fungal biocontrol agents, and also harassing them in managing pathogens in soil by both individual and in combination.

To evaluate the effectiveness, certain experimental setup has been followed,

Treatment; the treatment with various aspects that include control with healthy seeds inoculated seeds with pathogens, and seeds treated with chemical fungicides or biological control agents (both individually and in combinations).

Planting conditions: seedlings were transported into pots, and the requirement of pots per treatment and seeds per pot depended upon the experiment.

Inoculum preparation:

Biological agents were grown and prepared in specialized conditions for the experiment.

Disease assessment:

They are categorized into four divisions as follows:

Pre-emergence damping off: Evaluated 15 days after planting

Post-emergence damping off: Monitored 30 days after planting

Survival rate: Recorded 60days after planting

Root rot severity: Visually assessed on the level 0 to 5

Biochemical analysis:

Photosynthetic pigments: leaves have been extracted and analyzed spectrophotometrically after sowing on day 40

Enzyme activities: assessing polyphenol oxidase and peroxidase activities from root sample on day 15

Total phenolic content: on day 15 measure using a specific reagent

Lipid peroxidation: measured by malondialdehyde concentration on day 60

Membrane permeability: on Day 60 measured through electrolyte leakage percentage

Total soluble sugar: Determined by refractometer

Total carbohydrates and protein: they are measured using standard biochemical tests (Molisch's test and biuret test- general method to test carbohydrates and protein)

Treatment of host with biological control agents may significantly increase the levels of phenol, peroxidase, and polyphenol oxidase, and also in managing pathogen-induced plant disease.

Anatomical analysis:

Infection causes tissue damage and lysis in roots. On day 40, root samples are subjected to histological examination by analyzing root anatomy following micro technique procedures such as fixation, dehydration, embedding, sectioning, and staining. The thickness of the section, cortex, vascular cylinder, and diameter of xylem vessels were measured and recorded. By this there is a higher chance for triple combination treatment in improving root structure, increasing the thickness of root sections, cortex,

vascular cylinder, and xylem vessel diameter. In the same way, synergistic bilateral treatment may also contribute to increasing root thickness and xylem vessel diameter.

CONCLUSION:

The study reveals the role of beneficial microbes in enhancing soil fertility, promoting plant growth and also providing biocontrol against pathogens. By evaluating various microbial function microbial function, the potential of microorganism to support sustainable agriculture practices is evident. By this beneficial interaction, particularly in synergistic interaction among microbial community not only improve plant health and sustainability but also serve has alternatives for chemical pesticides. These microbes as an alternative to chemical pesticide, aligning with the increasing demand for ecofriendly agriculture practices. Thus, this emphasizes the importance of harnessing microbial interaction by synergistic effect in the control of disease and has a huge impact in creating eco-friendly environment.

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CHAPTER – 14

BOTANICALS IN INTEGRATED DISEASE MANAGEMENT (IDM): EFFICACY AND MECHANISM

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Abstract

Botanicals, derived from plants, play a crucial role in Integrated Disease Management (IDM) by offering sustainable and eco-friendly alternatives to synthetic chemicals. This chapter explores the efficacy of various botanical agents in managing plant diseases and elucidates their mechanisms of action. It covers the types of botanicals used, including essential oils, extracts, and plant derivatives, and discusses their integration into IDM strategies. The chapter examines the biological activity of these botanicals, their impact on pathogen suppression, and their role in enhancing plant resistance. Future prospects and challenges associated with the use of botanicals in IDM are also addressed, providing a comprehensive overview of their potential in sustainable agriculture.

1. Introduction

Definition and Importance of Integrated Disease Management (IDM)

Integrated Disease Management (IDM) refers to a holistic approach to controlling plant diseases through the judicious combination of biological, cultural, mechanical, physical, and chemical methods. Rather than relying solely on chemical pesticides, IDM emphasizes sustainable and environmentally sound strategies to minimize disease incidence and reduce economic losses. This approach not only delays the development of resistance in pathogens but also reduces pesticide residues in food and the environment, promoting long-term agricultural sustainability**.**

Overview of Botanicals in Plant Disease Management

Botanicals are plant-derived compounds or extracts used to manage pests and diseases. These include neem, garlic, turmeric, ginger, pungamia, and many others. They possess antifungal, antibacterial, antiviral, and insecticidal properties. In recent years, botanicals have gained increasing attention as eco-friendly alternatives to synthetic pesticides, particularly within organic

and sustainable farming systems. Their biodegradability, low toxicity to non-target organisms, and compatibility with other control methods make them ideal components in IDM programs.

Scope and Objectives

This chapter aims to explore the role of botanicals in the framework of IDM, providing insights into their mechanisms, effectiveness, and practical application in the field. Key objectives include:

- To understand the principles and components of IDM.
- To examine the types and sources of botanicals used in plant disease control.
- To assess the efficacy, advantages, and limitations of botanicals in integrated approaches.
- To highlight case studies and research findings supporting their use.

2. Types of Botanicals Used in IDM

2.1. Essential Oils

Common Essential Oils and Their Sources

Essential oils are volatile, aromatic compounds extracted from various parts of plants such as leaves, seeds, bark, or flowers. Notable examples include:

- **Neem oil** (*Azadirachta indica*): Rich in azadirachtin and used as a broad-spectrum antifungal and insecticidal agent.
- **Garlic oil** (*Allium sativum*): Contains sulfur compounds like allicin with strong antimicrobial activity.
- **Eucalyptus oil** (*Eucalyptus globulus*): Possesses antifungal and bactericidal properties.
- **Cinnamon oil** (*Cinnamomum zeylanicum*): Contains cinnamaldehyde, effective against fungal pathogens.

Applications and Effectiveness

Essential oils are typically used as sprays or seed treatments. They inhibit spore germination, disrupt cell membranes, and interfere with metabolic processes of pathogens. Due to their lipophilic nature, they easily penetrate plant and pathogen surfaces. Their **broad-spectrum activity**, **biodegradability**, and **low toxicity** to non-target organisms make them ideal for use in IDM.

2.2. Plant Extracts

Types of Extracts

Plant extracts are prepared using different solvents, depending on the nature of active compounds:

- **Aqueous extracts**: Simple water-based extractions, often used in traditional practices.
- **Ethanolic or methanolic extracts**: More efficient at extracting phenolics, flavonoids, and alkaloids.
- **Oil-based extracts**: Useful for lipid-soluble components.

Examples and Efficacy

- **Aloe vera**: Aqueous extracts show antifungal activity against *Alternaria* and *Fusarium* species.
- **Ginger extract** (*Zingiber officinale*): Contains gingerol and shogaol, effective against *Rhizoctonia solani* and *Pythium* spp.
- **Turmeric extract** (*Curcuma longa*): Rich in curcumin, known for antifungal and antibacterial properties.
- **Tulsi extract** (*Ocimum sanctum*): Exhibits broad-spectrum antimicrobial action.

These extracts act by inhibiting pathogen growth, disrupting cellular integrity, or inducing systemic resistance in the host plant.

2.3. Plant Derivatives

Plant-Based Compounds

Certain purified compounds from plants are particularly potent in managing plant pathogens. Key classes include:

- **Alkaloids**: Nitrogen-containing compounds (e.g., berberine, quinine) with strong antimicrobial effects.
- **Saponins**: Glycosides that disrupt fungal membranes (e.g., from *Sapindus* or *Dioscorea* species).
- **Phenolics and Flavonoids**: Found in many plants, they possess antioxidant and antimicrobial activity.
- **Tannins**: Polyphenolic compounds that inhibit enzyme activity in pathogens.

Mechanisms of Action

Plant derivatives act via:

- Cell membrane disruption
- Inhibition of spore germination

- Enzyme inhibition
- Oxidative stress induction

  Some also trigger induced systemic resistance (ISR) or systemic acquired resistance (SAR)**,** boosting the plant's internal defenses against a range of pathogens.

3. Efficacy of Botanicals in Disease Management

3.1. Biological Activity

Antifungal, Antibacterial, and Antiviral Properties

Botanicals contain a wide array of biologically active compounds, including alkaloids, flavonoids, terpenoids, phenolics, and essential oils, which exhibit potent antifungal, antibacterial, and antiviral properties.

- **Antifungal**: Neem (*Azadirachta indica*) extracts disrupt the mycelial growth of pathogens like *Fusarium oxysporum*, *Rhizoctonia solani*, and *Alternaria solani* by altering cell wall synthesis and membrane permeability.
- **Antibacterial**: Garlic (*Allium sativum*) and turmeric (*Curcuma longa*) extracts inhibit *Xanthomonas* spp. and *Pseudomonas syringae* through allicin and curcumin-mediated oxidative stress.
- **Antiviral**: Extracts from *Ocimum sanctum* (Tulsi) and *Eclipta alba* have shown inhibition of viral replication in *Tobacco mosaic virus (TMV)* infected plants.

Comparative Effectiveness with Synthetic Chemicals

While synthetic fungicides often exhibit faster action, many botanicals match or exceed them in long-term efficacy, particularly under integrated application:

- Botanical formulations like neem oil have been shown to reduce disease incidence by up to 60–80% in crops such as tomato and brinjal.
- In comparative trials, botanical sprays showed similar effectiveness to mancozeb and carbendazim in controlling foliar diseases, with the added benefit of lower environmental risk and residue-free produce.

3.2. Field Studies and Trials

Case Studies Demonstrating Efficacy in Various Crops

Numerous field experiments validate the practical effectiveness of botanicals:

- **Rice**: Application of *Trichoderma* and neem-based products significantly controlled sheath blight and blast.

- **Tomato**: Aqueous extracts of garlic and ginger reduced early blight severity caused by *Alternaria solani*.
- **Chilli**: Use of Pongamia oil and tulsi extract showed significant suppression of *Colletotrichum capsici* and *Phytophthora capsici*.
- **Groundnut**: Botanical sprays were effective against *Puccinia arachidis* and *Cercospora arachidicola*.

Success Stories and Limitations

Successes:

- India's National Horticulture Mission has promoted neem and pongamia products for widespread use in horticultural disease management.
- Organic farming projects in Europe and Africa incorporate plant extracts as a staple for crop protection with excellent community-level outcomes.

Limitations:

- Variability in active ingredient concentration depending on plant source, extraction method, and environmental conditions.
- Shorter residual activity compared to synthetic fungicides.
- Need for repeated applications, increasing labor and time requirements.
- Limited standardization and registration of botanical formulations in many regions.

3.3. Integration into IDM

Role in Reducing Chemical Use

Botanicals help reduce the dependency on synthetic fungicides, minimizing environmental pollution and resistance development. Their inclusion in IDM strategies supports:

- Lower chemical residue levels in produce.
- Improved soil microbial health, due to the non-toxic nature of most botanical compounds.
- Increased consumer acceptance of organically grown products.

Synergistic Effects with Other IDM Components

Botanicals often exhibit synergistic effects when combined with:

- Biocontrol agents (e.g., *Trichoderma*, *Pseudomonas* spp.)
- Cultural practices (e.g., crop rotation, sanitation)
- Reduced-dose chemical fungicides

4. Mechanisms of Action

4.1. Mode of Action against Pathogens

Disruption of Pathogen Cell Walls

Many botanicals contain compounds that directly disrupt the structural integrity of fungal and bacterial pathogens:

- Essential oils (e.g., thymol from thyme, eugenol from clove) permeabilize cell membranes, leading to leakage of cellular contents.
- Saponins, found in plants like *Quillaja saponaria*, integrate into lipid bilayers, causing pore formation and lysis of fungal cells.
- Alkaloids and phenolics destabilize pathogen cell walls by interfering with polysaccharide cross-linking, weakening their structure.

Inhibition of Pathogen Enzyme Systems

Botanical compounds interfere with vital enzymatic functions:

- Flavonoids and tannins inhibit enzymes such as cellulase, protease, and chitinase, which are essential for pathogen invasion and colonization.
- Curcumin and allicin are known to block respiratory chain enzymes, leading to metabolic failure in pathogens.

These mechanisms reduce pathogen viability and reproductive capacity, lowering the severity of disease outbreaks.

4.2. Enhancement of Plant Resistance

Induction of Systemic Acquired Resistance (SAR)

Several botanicals activate the plant's systemic defense mechanisms:

- Application of neem or ginger extracts can induce SAR, marked by the accumulation of pathogenesis-related (PR) proteins and the expression of defense genes.
- Compounds such as salicylic acid analogs, naturally found in certain plant extracts, are key in triggering SAR pathways.

Modulation of Plant Defense Responses

Botanicals can prime plants for enhanced response to pathogen attack:

- Phytoalexin production is stimulated by exposure to extracts from *Ocimum sanctum* and *Azadirachta indica*.
- Botanical treatments have been shown to modulate hormone signaling networks involving jasmonic acid, ethylene, and abscisic

acid, optimizing the plant's defense strategies without compromising growth.

4.3. Environmental Interactions

Influence on Soil Microbiome and Ecosystem Health

Unlike many synthetic pesticides, botanicals often support a favorable soil microbial environment:

- Repeated application of neem or pongamia oil has been observed to promote beneficial microbes such as *Trichoderma spp., Bacillus subtilis*, and mycorrhizal fungi.
- These interactions improve soil nutrient cycling, disease suppression, and plant growth promotion.

Impact on Non-target Organisms

Botanicals generally have low toxicity toward non-target organisms, including pollinators, natural enemies of pests, and soil fauna:

- For example, citronella and clove oil have limited impact on *Apis mellifera* (honeybees) and *Coccinellidae* (lady beetles).
- However, higher concentrations or non-standardized formulations may occasionally affect aquatic life or arthropod diversity, highlighting the importance of dose regulation.

5. Challenges and Future Directions

5.1. Standardization and Quality Control

Variability in Botanical Composition and Efficacy

One of the major constraints in botanical use is inconsistency in the chemical composition of plant-derived products:

- Environmental factors, such as soil type, climate, and plant maturity, significantly affect the concentration of bioactive compounds.
- Variability in extraction methods (aqueous, ethanolic, and supercritical) can result in formulations with inconsistent efficacy.
- Such inconsistencies hinder reproducibility in field trials and limit farmers' confidence in botanical products.

Methods for Standardizing and Ensuring Quality

To ensure consistent performance:

- Chromatographic profiling (e.g., HPLC, GC-MS) is being employed to identify and quantify active constituents.
- Establishing Good Manufacturing Practices (GMP) and standard protocols for collection, drying, and extraction is essential.

- Development of bioassays and quality control markers can help in regulating the potency and safety of commercial formulations.

5.2. Regulatory and Safety Issues

Compliance with Agricultural Regulations

Despite their natural origin, botanicals must comply with national and international agricultural regulations:

- In many countries, registration of botanical pesticides requires similar documentation as synthetic products, including efficacy, toxicity, and residue data.
- The lack of specific guidelines for botanicals often leads to delayed approvals and limited commercial availability.

Safety Considerations for Plants and Human Health

Though generally considered safer than chemicals, botanicals are not inherently risk-free:

- Over application or misapplication can lead to phytotoxicity, harming the crop itself.
- Some plant extracts contain toxic or allergenic compounds that may affect handlers or consumers if not properly tested.
- There is also a need for ecotoxicological evaluations to assess long-term environmental impacts, particularly on aquatic organisms and beneficial insects.

5.3. Future Research and Innovations

Emerging Botanicals and Their Potential

Research is expanding into lesser-known medicinal and wild plants for novel antimicrobial compounds:

- Species such as *Withania somnifera*, *Zingiber zerumbet*, and *Eclipta prostrata* show promise in preliminary screenings.
- Ethnobotanical surveys and traditional knowledge systems can help identify new leads for botanical pesticide development.

Advances in Extraction and Formulation Technologies

Technological improvements are driving innovation:

- Nano-formulations of botanical extracts offer improved stability, solubility, and targeted delivery.
- Microencapsulation and emulsification techniques are being used to enhance shelf-life and field efficacy.

- Integration with biopolymers or carriers (e.g., chitosan, alginate) may improve the controlled release of active ingredients and reduce degradation.

6. Conclusion

Botanicals have emerged as vital components of Integrated Disease Management (IDM), offering a natural, eco-friendly alternative to synthetic agrochemicals. Derived from a wide array of medicinal and aromatic plants, botanicals such as essential oils, plant extracts, and bioactive derivatives demonstrate significant antifungal, antibacterial, and antiviral activities. Their mechanisms—ranging from direct pathogen inhibition to the enhancement of host plant resistance—make them uniquely suited for integrated approaches that reduce reliance on chemical inputs.

Numerous field trials and case studies have confirmed the efficacy of botanicals across various crop systems. When combined with cultural, biological, and mechanical strategies, botanicals contribute to holistic disease suppression and improved agroecosystem health. Additionally, their generally low toxicity to non-target organisms and biodegradability make them compatible with the principles of sustainable and organic agriculture.

However, for botanicals to fulfill their full potential in IDM, challenges such as variability in composition, lack of standardization, regulatory hurdles, and limited shelf-life must be addressed. Advancements in extraction, formulation technologies, and quality control protocols will play a key role in overcoming these limitations.

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CHAPTER – 15

NON-FUNGICIDAL MANAGEMENT TECHNIQUES IN AGRICULTURE

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Abstract

As agriculture increasingly seeks sustainable and eco-friendly practices, non-fungicidal management techniques have emerged as critical strategies for disease control. This chapter explores a range of non-fungicidal approaches to managing plant diseases, focusing on integrated pest management (IPM), cultural practices, biological control, and resistant crop varieties. By analyzing these methods, we aim to provide a comprehensive overview of how they contribute to reducing reliance on chemical fungicides, enhancing crop health, and promoting environmental sustainability. Each technique is discussed with examples, benefits, limitations, and practical applications in various agricultural contexts.

1. Introduction

1.1. Background and Importance of Non-Fungicidal Techniques

Plant diseases caused by fungi, bacteria, viruses, and other pathogens pose a significant threat to global agricultural productivity and food security. Traditionally, fungicides have been the mainstay of disease management. However, growing concerns about fungicide resistance**,** chemical residues**,** environmental degradation**,** and human health risks have accelerated the need for sustainable, eco-friendly alternatives. Non-fungicidal techniques offer a promising approach to disease management without relying heavily on synthetic chemicals. These include biological control agents, cultural practices, resistant crop varieties, botanicals, and physical methods**,** which work synergistically to suppress disease incidence and promote crop health.

1.2. Overview of Plant Diseases and Their Impact on Agriculture

Plant diseases impact every stage of crop growth, leading to yield losses, reduced quality, and economic hardship for farmers. Common diseases such as late blight in potato, rusts in wheat, and wilt in pulses can decimate entire

fields if not effectively managed. Moreover, the emergence of new pathogen strains and climate-induced shifts in disease dynamics further complicate disease control strategies. In this context, integrated approaches that include non-fungicidal methods are increasingly being emphasized in Integrated Disease Management (IDM) programs.

2. Integrated Pest Management (IPM)

2.1. Principles of IPM

The fundamental principles of IPM revolve around preventing pest outbreaks**,** minimizing environmental harm**,** and ensuring economic viability**.** Core principles include:

- **Ecological Balance**: Understanding the interactions between pests, natural enemies, and the environment.
- **Threshold-Based Interventions**: Initiating control measures only when pest populations exceed economically damaging levels.
- **Integration of Methods**: Combining cultural, biological, mechanical, and chemical control tactics to achieve sustainable pest suppression.
- **Monitoring and Evaluation**: Continuously assessing pest populations and control outcomes to adapt strategies as needed.

2.2. Components of IPM: Monitoring, Thresholds, and Decision Making

IPM relies on a systematic framework for decision-making based on real-time data:

- **Pest Monitoring and Surveillance**: Regular field scouting, use of pheromone traps, light traps, and visual inspections help determine pest population dynamics.
- **Economic Thresholds**: Defined as the pest population level at which control measures should be applied to prevent economic loss. These vary by crop, pest species, and market conditions.
- **Decision-Making Tools**: Forecasting models, pest population databases, and extension advisories guide timely and appropriate interventions.
- **Record-Keeping and Evaluation**: Documentation of pest incidence and control efficacy is crucial for continuous improvement of IPM strategies.

2.3. Case Studies: Successful IPM Programs in Various Crops

a) Rice (Oryza sativa)

In India and Southeast Asia, IPM in rice involves alternate wetting and drying irrigation**,** biological control with *Trichogramma japonicum*, and judicious use of insecticides only after surpassing ETLs. This has reduced pesticide usage by over 50% in several regions.

b) Cotton (*Gossypium* spp.)

In Bt cotton fields, IPM strategies like use of trap crops (e.g., marigold for *Helicoverpa* spp.)**,** release of egg parasitoids, and pest-resistant cultivars have contributed to improved yields and reduced chemical residues.

c) Vegetables (e.g., tomato, brinjal)

Implementation of **s**ticky traps**,** botanical sprays (neem oil)**,** and use of net houses in vegetable cultivation has been effective in controlling whiteflies and fruit borers while preserving pollinators and beneficial insects.

2.4. Benefits and Limitations

Benefits:

- Reduced chemical pesticide usage**,** leading to lower residues in food and environment.
- Protection of natural enemies**,** enhancing biological control.
- Sustainability and long-term effectiveness of pest management.
- Improved profitability due to reduced input costs and better market access for residue-free produce.

Limitations

- Knowledge- and labor-intensive**:** requires scouting, threshold setting, and technical know-how.
- Higher initial costs and time requirements**,** deterrents especially for smallholders.
- Variability in effectiveness**,** depending on pests, climate, and adoption rates. Some invasive pests may only be partially managed.
- Collective action needed**,** as pest control is more effective across communities or landscapes.
- Policy and institutional challenges**,** including fragmented adoption, mixed regulations, and weak extension support.

3. Cultural Practices

3.1. Crop Rotation and Diversification

Crop rotation involves the sequential planting of different crop species in the same field over time. It helps in:

- Breaking the life cycle of soil-borne pathogens and pests.
- Reducing the inoculum load in the soil (e.g., rotating cereals with legumes to manage Fusarium wilt).
- Enhancing soil microbial diversity**,** which naturally suppresses pathogens.

Crop diversification—growing multiple crops or intercrops—adds ecological complexity, reduces disease spread, and enhances resilience to disease outbreaks**.**

3.2. Soil Management and Fertility

Healthy soil is critical to disease suppression. Practices include:

- Maintaining organic matter through compost and green manure.
- Balanced fertilization to prevent stress-related susceptibility.
- Drainage improvement to reduce diseases like Pythium and Phytophthora.
- Soil solarization (covering moist soil with transparent plastic) to reduce soil-borne pathogens and weed seeds.

Proper soil management boosts plant immunity, supports beneficial microorganisms, and prevents the buildup of harmful pathogens.

3.3. Planting Dates and Density Adjustments

Adjusting planting dates helps avoid peak disease periods. For instance:

- Early sowing of wheat escapes rust epidemics.
- Late planting of rice reduces incidence of bacterial leaf blight in certain regions.

Modifying plant spacing and density can:

- Improve airflow**,** reducing humidity that favors pathogens like downy mildew and powdery mildew.
- Minimize plant-to-plant disease spread.

These adjustments are crop- and region-specific**,** often requiring local adaptation.

3.4. Case Studies: Cultural Practices in Disease Management

- **Potato Late Blight (India)**: Early planting and wide spacing in high-altitude regions reduce Phytophthora infestations.
- **Maize–Legume Rotation (Africa)**: Suppresses soil-borne diseases like root rot and reduces Striga weed incidence.

- **Tomato Wilt Control (Philippines)**: Rotating with non-host crops like onion significantly reduces *Ralstonia solanacearum*.

Such examples demonstrate that cultural practices, when customized to crop ecology and pathogen behavior, can yield measurable disease control results.

3.5. Benefits and Limitations

Benefits

- Low-cost, farmer-friendly, and compatible with organic systems.
- Reduces dependence on chemical fungicides.
- Improves overall soil and plant health.
- Enhances long-term agricultural sustainability**.**

Limitations

- Often slow-acting and less immediately visible than chemical treatments.
- Require seasonal planning and sometimes detailed knowledge of local disease cycles.
- May not provide complete control in high disease pressure environments.
- Effectiveness can vary due to climatic or soil variability**.**

4. Biological Control

4.1. Introduction to Biological Control Agents

Biological control, or biocontrol, uses beneficial organisms to combat pests and pathogens in cropping systems. These organisms can act directly by attacking pests or indirectly by enhancing plant resistance or outcompeting harmful microbes. The major goals of biocontrol are to:

- Reduce pest and disease incidence without harming the environment.
- Minimize chemical pesticide use.
- Promote biodiversity and natural ecological balance.

4.2. Types of Biological Control: Predators, Parasitoids, and Pathogens

a) Predators

Predators are organisms that consume multiple prey in their lifetime. Examples:

- *Ladybird beetles* (*Coccinella septempunctata*) – feed on aphids.
- *Lacewing larvae* – feed on whiteflies, thrips, and mites.

b) Parasitoids

Parasitoids are insects whose larvae develop inside or on a host, ultimately killing it. Examples:

- *Trichogramma* spp. – parasitize eggs of lepidopteran pests.
- *Aphidius colemani* – attacks aphids.

c) Pathogens

These include bacteria, fungi, viruses, and nematodes that infect and kill pests or pathogens. Examples:

- *Bacillus thuringiensis* (Bt) – targets caterpillar larvae.
- *Trichoderma harzianum* – suppresses fungal pathogens like *Rhizoctonia*, *Fusarium*.
- *Beauveria bassiana* – entomopathogenic fungus against whiteflies and beetles.

4.3. Application Methods and Strategies

Biological control can be applied in several ways:

- **Inoculative Release**: Small initial release allowing the agent to multiply (e.g., *Trichoderma* in seed treatment).
- **Inundative Release**: Large-scale application for quick control (e.g., spraying Bt formulations).
- **Conservation Biocontrol**: Creating favorable conditions to promote natural enemies (e.g., hedgerows, nectar plants).
- **Seed and Soil Treatment**: Beneficial microbes applied to seeds or soil to establish early protection.
- **Foliar Application**: Used for fungal or bacterial biopesticides on leaves.

4.4. Case Studies: Biological Control in Practice

- **Cotton (India)**: *Trichogramma chilonis* released to control *Helicoverpa armigera*, reducing pesticide sprays.
- **Tomato and Chilli Crops**: *Pseudomonas fluorescens* used as a foliar spray to suppress leaf spot and bacterial wilt.
- **Banana Wilt Management**: *Trichoderma viride* applied to planting pits reduces *Fusarium oxysporum* wilt incidence.
- **Greenhouse IPM (Europe)**: Use of *Encarsia formosa* to manage whiteflies in tomato and cucumber greenhouses.

4.5. Benefits and Limitations

Benefits

- Environmentally safe and biodegradable.
- Compatible with organic farming and IDM/IPM practices.
- Target-specific**,** minimizing harm to non-target organisms.
- Supports ecological balance and biodiversity.

Limitations

- Slower action compared to chemical pesticides.
- Environmental dependency—effectiveness varies with humidity, temperature, and crop type.
- Limited shelf life and challenges in large-scale storage and transportation.
- Requires farmer awareness and training **f**or proper application.

5. Resistant Crop Varieties

5.1. Development and Selection of Resistant Varieties

Resistant varieties are developed through conventional breeding, molecular breeding, or genetic engineering. These processes involve:

- Screening germplasm for natural resistance traits.
- Hybridization and selection for resistance in progeny.
- Marker-assisted selection (MAS) to accelerate breeding.
- Transgenic approaches to introduce specific resistance genes (e.g., Bt, CRISPR-edited resistance).

Public research institutions and private seed companies collaborate globally to develop region-specific resistant varieties adapted to local agro-climatic conditions.

5.2. Mechanisms of Resistance

Plant resistance to pathogens may be:

a) Structural Resistance

- Thick cuticles, waxy layers, or trichomes that act as physical barriers.
- Example: Hairy leaf surfaces in cotton reduce aphid colonization.

b) Biochemical/Physiological Resistance

- Production of antimicrobial compounds (phytoalexins, phenolics).
- Activation of pathogenesis-related (PR) proteins**.**
- Hypersensitive response (HR) – rapid localized cell death to restrict pathogen spread.

c) Genetic Resistance

- **Vertical resistance (monogenic)**: Specific and strong, but may be overcome quickly by evolving pathogens.
- **Horizontal resistance (polygenic)**: Broad-spectrum and durable, though less intense.

Understanding these mechanisms allows breeders to develop varieties that are both effective and long-lasting in the field.

5.3. Examples of Resistant Varieties in Different Crops

Crop	Disease	Resistant Variety
Rice	Bacterial leaf blight	IRBB21, Samba Mahsuri (sub1)
Wheat	Leaf and stem rust	HD 2967, DBW 303
Tomato	Tomato mosaic virus (ToMV)	Arka Meghali, Pusa Rohini
Brinjal (Eggplant)	Shoot and fruit borer, Phomopsis blight	Arka Keshav, Pusa Purple Cluster
Potato	Late blight	Kufri Jyoti, Kufri Surya
Cotton	Bollworms	Bt cotton hybrids
Chilli	Anthracnose, leaf curl	Arka Lohit, Kashi Anmol

These varieties are widely adopted due to their yield stability and lower disease incidence.

5.4. Benefits and Limitations

Benefits

- Reduced need for fungicides and pesticides**.**
- Environmentally friendly and compatible with organic systems**.**
- Cost-effective and easy to integrate with other IDM practices.
- Improves yield stability even under high disease pressure.

Limitations

- Resistance may break down over time due to pathogen evolution (especially with vertical resistance).
- Time-consuming and expensive to breed for complex traits.
- May have trade-offs with yield or quality in some varieties.
- Resistance is often pathogen-specific, requiring combination with other control measures.

6. Combining Non-Fungicidal Techniques

6.1. Integrated Approaches for Optimal Disease Management

Combining non-fungicidal strategies requires thoughtful planning and understanding of pathogen biology, host susceptibility, and environmental factors. Key integration strategies include:

- Use of resistant varieties along with botanical sprays (e.g., neem oil) for layered protection.
- Biological control agents (*Trichoderma*, *Pseudomonas*) applied **to** resistant cultivars for enhanced root protection.
- Cultural practices (crop rotation, spacing, sanitation) used with botanicals **t**o reduce initial inoculum load.
- Soil amendments (e.g., compost, biochar) integrated with beneficial microbes to improve plant health and suppress pathogens.
- Monitoring and threshold-based actions to optimize timing of botanical and biological applications.

Example: In tomato cultivation, combining resistant varieties (e.g., resistant to *Ralstonia solanacearum*) with soil application of *Trichoderma harzianum* and neem cake resulted in significantly reduced wilt incidence compared to using a single method.

6.2. Case Studies: Successful Integration of Techniques

Case Study 1: Rice Blast Management

- **Practices used**: Resistant variety (IR64), seed treatment with *Pseudomonas fluorescens*, cultural control (timely transplanting), and use of botanical spray (garlic extract).
- **Outcome**: Over 60% reduction in disease incidence, enhanced grain yield.

Case Study 2: Tomato Early Blight Control

- **Practices used**: Crop rotation with cereals, use of resistant cultivar, mulching, and foliar sprays of neem oil.
- **Outcome**: Consistent reduction in blight severity and improved fruit quality.

Case Study 3: Brinjal Fruit and Shoot Borer & Phomopsis Blight

- **Practices used**: Bt brinjal hybrids, intercropping with marigold, release of *Trichogramma*, and seed treatment with *Trichoderma*.
- **Outcome**: Decreased pest and disease infestation, reduced chemical sprays, and better marketable yield.

6.3. Benefits and Limitations

Benefits

- Reduced chemical use, leading to lower residues and safer food.
- Sustainable disease suppression by delaying pathogen resistance.
- Encourages ecological balance and promotes beneficial organisms.
- Often cost-effective over the long term.
- Aligns with organic and climate-resilient farming goals**.**

Limitations

- Requires knowledge and skill to implement effectively.
- Success depends on timing, sequencing, and compatibility of techniques.
- Slower initial results compared to chemical fungicides.
- Limited availability of quality biocontrol products or resistant varieties in some regions.

7. Conclusion

7.1. Summary of Key Points

Non-fungicidal strategies offer a sustainable and ecologically sound alternative to chemical disease control. The key components discussed include:

- **Integrated Pest Management (IPM)**, which brings together cultural, mechanical, biological, and behavioral strategies to minimize disease without relying heavily on chemicals.
- **Botanicals**, such as essential oils and plant extracts, which show promising antifungal and antibacterial activity.
- **Biological control agents**, including beneficial fungi, bacteria, and viruses, that naturally suppress plant pathogens.
- **Resistant crop varieties**, which provide genetic defense against specific diseases and reduce the need for chemical interventions.
- The **integration of these techniques**, which enhances efficacy, reduces environmental impact, and contributes to long-term disease suppression.

These strategies, when used in combination, form a robust foundation for sustainable and climate-smart agriculture.

7.2. Future Directions and Research Needs

While non-fungicidal approaches hold great promise, several challenges must be addressed to ensure widespread adoption:

- **Standardization and validation** of botanicals and biological agents under diverse agro-climatic conditions.
- **Development of next-generation resistant varieties** using advanced breeding and gene-editing technologies.
- **Long-term ecological studies** to assess the impact on soil health, biodiversity, and non-target organisms.
- **Innovative formulations and delivery systems** for botanical and microbial products to enhance field effectiveness.
- **Policy support and farmer training programs** to improve awareness, adoption, and practical implementation.

More interdisciplinary research is needed to optimize these strategies and adapt them to specific crops and regions.

7.3. Final Thoughts on Sustainable Disease Management

Non-fungicidal disease management is not just a response to the overuse of chemicals—it is a proactive strategy for building resilient agricultural systems. By integrating ecological principles, traditional knowledge, and modern science, farmers can manage diseases in ways that safeguard human health, environmental integrity, and long-term productivity.

As global agriculture faces the dual challenges of climate change and increasing food demand, the role of sustainable disease management practices particularly those that exclude synthetic fungicides will become increasingly vital.

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CHAPTER 16

AZOXYSTROBIN – A NEW FUNGICIDE MOLECULE FOR THE MANAGEMENT OF MAJOR DISEASES OF CHILLI

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Abstract

Studies were undertaken on the incidence of anthracnose and powdery mildew in chilli in Tamil Nadu, nature of causative agents, bio efficacy of Azoxystrobin 23 w/w against the pathogens of anthracnose and powdery, persistence of Azoxystrobin in plants, its compatibility with insecticides and bio control agents, residues of Azoxystrobin in the plants, effect of infection on leaf and fruit quality and role in induced systemic resistance

Introduction

Chilli (*Capsicum annum*) is an important vegetable cum spice crop in India.It accupies 792.1 thousand million ha with an yield of 122.3 million tones (Indian horticultural database 2011-2012) Chilli is affected by many fungal, bacterial and viral diseases. Among them, anthracnose and powdery mildew are the major diseases accounting for heavy yield losses. Anthracnose, caused by *Colletotrichum* spp. is a serious problem for chilli production in the tropics and subtropics worldwide (Montri *et al*., 2009) which causes qualitative and quantitative yield losses Mathur *et al*. (1972) reported a severe defoliation and reduction in size and number of chilli fruits due to powdery mildew and there was 25-30 per cent increase in yield with the use of fungicides. In the absence of effective fungicides, powdery mildews reduced yield by 55 per cent (Sivaprakasam *et al*., 1976).The yield loss ranging from 14.20 per cent to 19.60 per cent due to powdery mildew of chilli was reported by Gohokar and Peshney (1981).The literatures pertaining to the fungi associated with diseases, disease

management strategies using fungicides, persistence of fungicides in plants, their compatibility with insecticides and biocontrol agents and induction of systemic resistance are reviewed under in this chapter**.**

Chilli anthracnose

Fungi associated with anthracnose

Symptoms produced by anthracnose fungi

The disease produces symptoms on leaves, stem and fruits. The symptoms produced by *Colletotrichum* spp. have been described by several authors (Kannan *et al*., 1998; Rangaswami and Mahadevan, 2005; Ratanacherdchai *et al*., 2007). Symptoms of the disease include pre- and post-emergence damping-off, dieback of shoots, leaf spots and fruit rots either in the field or in storage. The disease symptoms appear mostly on ripened fruits and hence the disease is also called ripe-fruit rot. The symptom appears as circular and sunken spots with black margins and covered with a pinkish mass of fungal spores. At later stage the spots spread, forming concentric markings with dark fructifications representing the fungal acervuli. The fruits may drop prematurely, resulting in heavy loss in yield (Rangaswami and Mahadevan, 2005).

Chilli Powdery Mildew

Fungi associated with powdery mildew

The powdery mildew of chilli is predominantly caused by *Leveillula taurica* (Oreux nd Felix, 1963; Reuveni and Rotem, 1973; Dixon, 1978).

Symptoms produced by powdery mildew

Oreux and Felix (1963) reported that the powdery mildew of chilli caused by *L. taurica* resulted in severe defoliation, leading to reduction in size and number of fruits per plant. Reuveni and Rotem (1973) described the symptoms of disease on the underside of mature leaves as brown sites covered with whitish growth and shedding of even symptomless leaves. Blazques (1976) described that powdery mildew in chilli appears first on the older leaves and then progresses to younger leaves. Jharia *et al*. (1978) reported that the disease generally appears at the time of flowering and fruit formation. The diseased leaves curl, fruits remain smaller and plants ultimately dry.

***In vitro* evaluation of fungicides**

In vitro* evaluation of fungicides against *C.capsici

Motikkhaye (1983) reported that aureofungin and carboxin reduced the mycelia growth and conidial germination of *C.capsici*. Jeyalakshmi (1996) reported that carbendazim at 0.1 and 0.05 per cent and mancozeb at 0.2 per cent effectively reduced the mycelial growth and conidial germination of *C.capsici*. Gupta and Shyam (1996) evaluated the efficacy of seven fungicides in reducing sporangial production, metalaxyl combined with mancozeb was the most effective treatment in reducing the number of sporangia followed by cymoxanil alone and also in combination with mancozeb.

In vitro* evaluation of fungicides against *L.taurica

Gohokar and Peshney (1981) conducted *in vitro* evaluation of twelve fungicides against *L. taurica* of chilli. Sulphur dust, sultaf (Wettable sulphur) and karathane (dinocap) inhibited spore germination and dinocap and thiovit (Wettable sulphur) inhibited germtube elongation. Nawaz and Narayanasamy (1983) noticed karathane (0.05%), bavistin (0.1%), wettable sulpur (0.3%) and benlate (0.1 %) were found to be most effective, which totally inhibited the germination of conidia.

Field evaluation of fungicides against *C.capsici*

Thind and Jhooty (1987) found that spraying of nifolatan at 0.2 per cent followed by mancozeb 0.2 per cent gave economical control of *C. capsici* and *A. alternata* on chilli under Punjab conditions. Ebenezar and Alice (1996) reported that fruit rot of chilli was effectively controlled by spraying of mancozeb (0.2 per cent) followed by carbendazim (0.2 per cent) and copper oxychloride (0.2 per cent). Lewis-Ivey *et al*. (2004) conducted field trail with anthracnose susceptible cultivar ACX229 and with some cultivars of bell pepper

Field evaluation of fungicides against *L.taurica*

Mathur *et al*. (1972) found that, three sprays of 0.5 per cent elosal, 0.25 per cent sulkol, 0.2 per cent cosan, 0.5 per cent thiovit and sulphur dust at 25 lb / acre at fortnightly interval reduced the incidence of powdery mildew of chilli. They observed increase in yield by all the treatments especially in sulkhol. Moghal *et al*. (1977) opined that, all the sulphur fungicides (Sulforon dust, Sulforon spray, Cosan and Thiovit Sulphur tested against *Leveillula taurica* on capsicum lowered infection significantly and increased yield.

Compatibility studies

Compatible means capable of existing together in harmony (*i.e.,*) able to exist together with something also. Often it becomes necessary to mix two or three compatible pesticides (insecticide with fungicide) in a single preparation

which saves time and expenses. These two chemicals when mixed in a single preparation due to reaction form a compound, which differs from the parent .Use of incompatible chemicals may result in the undesirable effects. The compatibility in such cases may be physical compatibility and phytotoxicity.

Physical incompatibility

The chemicals when mixed change their physical form to one that is unstable and harmful for application. The indiscriminative use of potentially hazardous fungicides poses a serious threat to environment. The compatibility on beneficial organisms such as nitrogen fixers, residential antagonists and mycorrhizal fungi are the other advantages of the application of fungicides (Rodriguez- Kabana and Curl, 1980).

Varalakshmi *et al.* (2000) showed that the hexaconazole + monocrotophos (500 ml ha + 1000 mi ha) combination was highly effective over other treatments in reducing the powdery mildew incidence,thrips and flea beetle damage in grapes. Both the fungicide and insecticide were found to have synergistic effect and exerted high efficiency towards pests and diseases of grapes. Suganthy *et al*. (2010) Azoxystrobin, wettable sulphur, carbendazim is compatible with imidacloprid 17.8 % SL and results showed that the phytotoxic effects of these imidacloprid 17.8% SL at 25g a.i./ha with above fungicides on cotton, bhendi and chilli does not cause any phytotoxic symptoms such as injury to leaf tip and leaf surface, wilting, vein clearing, necrosis, epinasty and hyponasty

Compatibility of fungicides with bio control agents

Sendhil Vel *et al.* (2004b) reported that turbidometric assay showed that the bacterial biocontrol agents(*P.fluorescens* and *B.subtilis*) growth in azoxystrobin-amended broth was not affected and is perfectly compatible with bacterial biocontrol agents.

Katria *et al*. (2002) tested *Pseudomonas fluorescens* as seed treatment against R.solani in bean with the combination of Azoxystrobin, fluidioxanil (0.2%), pencyuron and tebuconazole (0.2%) found to be compatible. Utkhede and Koach (2002) suggested that azoxystrobin and *Bacillus subtilis* could be applied together as post inoculation spray against gummy stem blight of cucumber caused by *Didymella bryoniae* (Auersw.) Rehm. Leha and Venkataraman (2001) reported that carbendazim was very much compatible with *Pseudomonas fluorescens*. Significantly higher bacterial population was

obtained in 1000 ppm carbendazim amended King's B medium (KBM) than in 500 ppm amended KBM alone.

Phytotoxicity

In certain combinations, no chemical reaction is exhibited and the components by themselves are not injurious to plants, but the mixture proves to be phytotoxic to the plants (Paul MohanRoy, 1988).

Biochemical basis of resistance

Chlorophyll content

Chlorophyll is vital for photosynthesis, which allows plants to absorb energy from light. Chlorophyll molecules are specifically arranged in and around photosystems that are embedded in the thylakoid membranes of chloroplasts. In these complexes, chlorophyll serves two primary functions. The function of the vast majority of chlorophyll (up to several hundred molecules per photosystem) is to absorb light and transfer that light energy by resonance energy transfer to a specific chlorophyll pair in the reaction center of the photosystems.

Once the pathogen gains the entry into host, there was alteration in the normal mechanism of host. Kortekamp (2006) showed that the infection of powdery mildew in chilli resulted in reduction of photosynthesis with reduced plant vigour. Hence, taking these points in consideration the chlorophyll content was estimated for healthy and infected leaves. It was observed that chlorophyll content was reduced in infected leaves both in glasshouse and field conditions

Triadimefon and hexaconazole treatment increased the carotenoid content in tapioca leaves. Triadimefon treatment induced higher level of carotenoid content in cow pea (Gopi *et al.,* 1999). Similar results were observed in ketoconazole. (Abdul Jaleel *et al*., 2007a) and paclobutrazol (Abdul Jaleel *et al*., 2007b) in *Catharanthus roseus* treatment. Increased level of cytokinin particularly transzeatin and its riboside has been reported in sunflower cell suspension, rice, soybean and rape seedlings after uniconazole treatment and thus increased zeatin might be responsible for the increased synthesis of carotenoid in the plants. (Grossmann, 1992).

The estimation indicates the photosynthetic activity reduced after pathogen infection. Sanjay Guleria *et al*. (1997) reported that, changes in chlorophyll, sugar contents in powdery mildew of pea after infection showed

that decrease in chlorophyll (a,b and total) and reducing sugar content in the leaves of both resistant and susceptible cultivars, whereas the total and non reducing sugar contents increased in all the cultivars. The higher percentage increase in non reducing sugar content in resistant than susceptible cultivars indicates that, they may be involved in disease incidence

Phenols

The perusal of literature indicated no work has been done on the role of phenols in anthracnose disease in chilli and hence work on other crops are revieved.

Plant Phenolics are the well known antifungal, antibacterial and antiviral compounds (Vidhyasekaran, 1998a and Bennett and Wallsgrove, 1994). Phenols play an important role in determining resistance or susceptibility of a host to parasite infection (Vidhyasekaran, 1998a,b).

Capsaicin

Capsaicin is the active component of chili peppers, which are plants belonging to the genus *Capsicum* Capsaicin and several related compounds are called capsaicinoids and are produced as a secondary metabolite by chili peppers, probably as deterrents against certain fungi. Pure capsaicin is a hydrophobic, colorless, odorless, crystalline to waxy compound.

Presence of alkaloid capsaicin in chilli gives pungency nature to it (Cichewicz and Thorpe, 1996). Capsaicin not only makes chilli pungent or hot, but also inhibits the growth of many microorganisms. Prakasam (1983) reported that green chillli fruits contained more capsaicin than ripe susceptible fruits.

Ascorbic Acid

Ascorbic acid is a naturally occurring organic compound with antioxidant properties Ascorbic acid is one form vitamin C. Ascorbate typically reacts with oxidants of the reactive oxygen species, such as the hydroxyl radical formed from hydrogen peroxide. Ascorbic acid is an important component of the plant antioxidant system (Abdul Jaleel *et al*., 2008a). Ascorbate is one of the two major soluble antioxidants in chloroplast (Shao *et al.,* 2009). Ascorbate also has photo protective function because of its antioxidant capacity (Abdul Jaleel *et al*., 2009) Ascorbic acid (AA) is a very important reducing substrate for H2O2 detoxification in photosynthetic organisms (Jaleel *et al.,* 2007).

Abdul Jaleel *et al.* (2008b) found that application of triazole fungicides on yam plant showed increased chlorophyll, carotenoid, xanthophyll and also increased the antioxidant ascorbic acid. Biljana *et al.* (1993) reported that pepper plant treated with fungicides Previour N and Enovit M showed a 6 to 19% increase of ascorbic content in pepper compare to untreated control. Treatment with azoxystrobin retarded the rate of conversion of ascorbic acid into dehydroascorbic acid.

Lipid Peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene -CH2- groups that possess especially reactive hydrogens Lipid peroxidation refers to the oxidative degradation of lipids

Gomathinayagam *et al.* (2008) found that the lipid peroxidation in the leaf cells was inhibited by the triazole treatment. The inhibition of lipidperoxidation was higher in triadimefon and hexaconazole treated plants and it was 67.88 and 64.8 on 240 DAP. Triadimefon and hexaconazole treated tapioca plants showed a lower level of lipid peroxidation when compared to control. Malonoldialdehyde (MDA) a product of lipid peroxidation damages enzymes and plant membranes as observed in *Daucus carota* tubers (Gopi *et al*., 2007). The lipid peroxidation is a consequence of higheroxidative stress (Abdul Jaleel *et al*., 2008c). Uniconazole reduced the electrolyte leakage and MDA accumulation and consequently decreased the heat induced lipid peroxidation in rape plants Zhou and Leul, 1999.

Induced systemic resistance

Induced resistance is defined as an enhancement of the plant's defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimulation. The resulting elevated resistance due to an inducing agent upon infection by a pathogen is called Induced Systemic Resistance (ISR) or Systemic Acquired Resistance (SAR) (Hammerschmidt and Kuc, 1995). The induction of systemic resistance by rhizobacteria is referred as ISR, whereas that by other agencies is called SAR (Van Loon *et al*., 1998). Once resistance is induced it will afford non-specific protection against

pathogenic fungi, bacteria and viruses as well as against several insects and nematodes.

A large number of defense enzymes have been associated with ISR which include phenylalanine ammonia lyase, chitinase, β-1,3-glucanase, peroxidase, polyphenol oxidase, SOD, CAT, APX, lipoxygenase and proteinase inhibitors (Koch *et al*., 1992; Schneider and Ullrich, 1994; Ye *et al*., 1990).

Peroxidase (PO)

Peroxidases have been implicated in the regulation of plant cell elongation, phenol oxidation, polysaccharide cross-linking, IAA oxidation, cross linking of extension monomers, oxidation of hydroxyl–cinnamyl alcohols into free radical intermediates and wound healing (Vidhyasekaran *et al*., 1997). Bradley *et al*. (1992) reported that the increased PO activity has been correlated with resistance in many species including barley, cucurbits, cotton, tobacco, wheat and rice and these enzymes are involved in the polymerization of proteins and lignin or suberin precursors into plant cell wall, thus constructing a physical barrier that could prevent pathogen penetration of cell walls and movement through vessels

Anand (2002) reported an increased activity of peroxidase, in bacterized fruits of Chilli challenged with the pathogen. Ramamoorthy and Samiyappan (2001) studied the induction of defense related genes in *Pseudomonas fluorescens* treated chilli plants in response to infection by *Colletotrichum capsici.* They found that the level of phenolics and activity of phenylalanine ammonia lyase, polyphenol oxidase, ß-1, 3 glucanase, chitinase and peroxidase were higher in *P. fluorescens* treated plants challenged with the pathogen.

Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO or monophenol monooxygenase) is a tetramer that contains four atoms of copper per molecule, and binding sites for two aromatic compounds and oxygen. The enzyme catalyses the *o*-hydroxylation of monophenols (phenol molecules in which the benzene ring contains a single hydroxyl substituent) to *o*-diphenols (phenol molecules containing two hydroxyl substituents).

PPO usually accumulated upon wounding in plants. Biochemical approaches to understand PPO function and regulation are difficult, because

the quinonoid reaction products of PPO covalently modify and cross-link the enzyme. PPO can be induced *via* octadecanoid defense signal pathway (Constabel *et al*., 1995).

Sundaravadana *et al.* (2007b) reported that reduction of rice blast severity was mainly associated with induction of host defense mechanism by azoxystrobin. Increased the production of secondary metabolite – phenolic and lignification related enzymes, namely peroxidase, polyphenol oxidase and phenylalanine ammonia- lyase were observed in rice plants treated with azoxystrobin.

β-1,3-glucanases (PR2)

β – 1, 3 – glucanase activity has been suggested to have a role in plant defense against disease caused by fungi because their substrate β – 1, 3 – glucan is a major component of the cell walls of many fungi. The enzyme may also play a role in plant defense by releasing glucan fragments (elicitors), from fungi on plant cell walls that elicit phytoalexin accumulation in the plants

Evidence of β-1,3-glucanases (EC 3.2.1.6) in disease resistance was first reported by Kauffmann *et al*. (1987). In dicots, β-1,3-glucanase genes are considered to constitute a part of the general array of defense genes induced during pathogenesis (Mauch and Staehelin, 1989). Later, induction of β-1,3-glucanases was demonstrated in barley and other monocots like wheat, rice and sorghum in response to infection by the necrotrophic pathogen, *Bipolaris sorokiniana* (Jutidamrongphan *et al*., 1991). Daugrois *et al*. (1992) reported rapid induction of two β-1, 3-glucanases in the incompatible interaction between bean and *C. lindemuthianum.* Purified fungal elicitor also induced these enzymes in the bean host. Purified acidic β-1,3-glucanases from cucumber had antifungal activity against *C. orbiculare* (Ji and Kuc, 1996).

Phenylalanine ammonia lyase (PAL)

Phenylalanine ammonia lyase (PAL) is one of the most important enzymes responsible for the formation of phenolic compounds. It catalyses the conversion of L - Phenylalanine to *trans* - cinnamic acid. Cinnamic acid serves as a precursor for the biosynthesis of coumarins, isoflavanoids and lignin. These compounds play an important role in pest and disease resistance mechanism. Changes in

PAL activity accompanying fungal, viral and bacterial infections of plants have been reported.

PAL catalyzes the deamination of L-phenylalanine to trans-cinnamic acid which is the first step in the biosynthesis of large class of plant natural products based on the phenylpropane skeleton, including lignin monomers as well as certain classes of phytoalexins. PAL activity also generates precursors of lignin biosynthesis and other phenolic compounds that accumulate in response to pathogen infection (Klessig and Malamy, 1994). PAL is the key enzyme in inducing synthesis of salicylic acid (SA) which induces systemic resistance in many plants. In rice, ZB8 PAL gene was found to be induced by the elicitor treatment in rice cells (Li *et al*., 1993). The PAL gene was cloned and transgenic rice plants expressing PAL ZB8 showed systemic resistance against rice pathogens (Lamb *et al*., 1997).

Katz *et al.* (1998) found that benzothiadiazole (BTH) induced PAL genes, emphasizing an important role of defense response potentiation in acquired plant disease resistance.

In the probenazole treated and P.grisea inoculated leaves the activity of Peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and catecholmethyl transferase were higher than in the inoculated leaves. This indicated that the disease controlling mechanisms of probenazole was due to the host mediated defense reaction. (Davise and Ward, 1984; Hewit, 2001)

Chitinases (PR3, PR4, PR8 & PR11)

Chitinases are PR-proteins which hydrolyze chitin, major cell wall component constituents for 3-10% of higher fungi and cuticle of peritrophic membrane in insects. Chitinase cleave a bond between C1 and C4 of two consecutive *N*-acetyl glucosamine (GlcNAc) either by endolytic or exolytic mechanisms. A large number of plant chitinases have been purified and characterized which are endochitinases with molecular weights ranging from 25 to 36 kDa. The production of chitinases in plants has been suggested to be a part of their defense mechanism against fungal pathogens (Schlumbaum *et al*., 1986).

Anand (2002) reported an increased activity of chitinase in bacterized fruits of Chilli challenged with the pathogen. Ramamoorthy and Samiyappan (2001) studied the induction of defense related genes in *Pseudomonas fluorescens* treated chilli plants in response to infection by *Colletotrichum capsici.*

Scavengers of reactive oxygen species (ROS)

One of the biochemical changes occurring in plants subjected to various environmental stress conditions is the production of reactive oxygen species (ROS) such as superoxide radicals (O^{2-}), hydrogen peroxide, singlet oxygen and hydroxyl radicals (OH) (Iturbe- Ormaetxe *et al*., 1998; Cho and Park, 2000).

Various antioxidant enzymes such as CAT and PO eliminate H_2O_2. CAT found predominantly in peroxisomes dismutase H_2O_2 into H_2O and O_2, whereas PO decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and antioxidants (Sudhakar *et al*., 2001). Catalase and peroxidase are of particular interest because of their role in binding SA, which plays an important role in induced resistance (Anderson *et al.*, 1998). APX is primarily located in both chloroplasts and cytosol and eliminates peroxides by converting ascorbic acid to dehydroascorbate (Asada, 1992). As a member of the ascorbic acid glutathione cycle, APX is one of the most important enzymes playing a crucial role in eliminating toxic H_2O_2 from plant cells during biotic and abiotic stress (Foyer *et al*., 1994; Cho and In-Taek, 2003).

Lipoxygenase (LOX)

Plant cell membranes consist of mostly lipids and proteins. The polyunsaturated octadecanoid fatty acids, linoleic and linolenic acids are the common constituents of plant membranes (Shimura *et al*., 1983). When plants are infected by pathogens and insect pests various lipids breakdown to form the products including C_6 volatiles from linoleic acid and linolenic acid by sequential steps involving lipoxygenase (EC 1.13.11.12), hydroperoxide lyase and hydroperoxide hydralase in the so called lipoxygenase pathway (Croft *et al*., 1993). Plant LOX are membrane of a class of nonhaeme iron-containing dioxygenases that catalyze the addition of molecular oxygen to fatty acids containing a cis, cis-1,4-pentadiene system to give an unsaturated fatty acid hydroperoxide (Siedow, 1991). Linolenic acid is the substrate for LOX.

A comprehensive list of volatile emission signals and plant responses, broken down by plant species has been well compiled. These studies suggest that LOX are related to plant growth and development, senescence, plant defense against pathogens and insects and biosynthesis of regulatory molecules.

Isozyme studies

The application of protein analysis to the screening among variety of tissues and organisms are finding increasing use in research on population genetics and taxonomy. Since the initial investigations of Chang *et al*. (1962) on *Neurospora* species several investigators have examined the possibility of separating proteins or enzymes of fungi by electrophoresis in starch or acrylamide gels and using the result and patterns for differentiation (Meyer and Renard, 1969; Hall, 1971; Reddy and Stahamann, 1972; Scala *et al*., 1981). The first major contribution in isozymes was the development of starch gel electrophoresis by Smithies (1955). Hunter and Market (1957) showed that enzymes could be visualized directly on starch gels when stained with a specific histo-chemical stain and they proposed the term zymogram to refer to the strips in which enzymes location was demonstrated. Market and Muller (1959) introduced the term isozyme to describe multiple enzyme forms with similar or identical substrate specificity within the same organism.

Gel electrophoretic techniques of soluble proteins and enzymes have been applied to three distinct areas of plant disease research. Initially, those techniques were seen primarily as a potential powerful taxonomic tool with which it might be possible to clearly delinate specific or sub specific groups of different fungal pathogens. At the same time, these techniques maybe used for detailed biochemical aspects of host resistance and the effect of such pathogens on the metabolism of their hosts. Clare and Zentmyer (1966) and Gill and Powell (1968) whileworking with various combinations of six species of *Phytophthora* found consistent differences between the general protein pattern of the species studied.

Sako and Stahmann (1972) studied the multiple molecular forms of the enzymes (isoenzymes) from near isogenic barley lines inoculated with powdery mildew by polyacrylamide disc electrophoresis. Following the inoculation of the susceptible line the number of isoenzymes bands of acetylesterase, acidphosphatase, malatedehydrogenase succinate dehydrogenase and peroxidase were increased. New bands were not detected in extract of healthy tissue or mycelium and conidia from infected leaves. Their intensity was not reduced by removing mycelium and conidia before preparing extracts. Only peroxidase band appeared within one day after inoculation. Further, they opined that, all the changes in enzymes could not be explained by a simple contribution from the fungus.

Serious consideration should be given to the effect of interaction between host and parasite isozymes. Hasabnis (1998) studied the peroxidase and polyphenoloxidase enzymes in wheat and leaf rust interaction. He reported that activity of both the isozymes was higher in response to inoculation of the pathogen which was expressed by producing more number of bands. Patel *et al*. (2001) employed polyacrylamide gel electrophoresis technique for identification of varieties of chilli, tomato, brinjal and bhendi. Zymogram of each genotype showed that the soluble protein banding pattern was more effective for cultivar identification in chilli tomato, brinjal and bhendi. However, in brinjal both protein and peroxidase isozymes could be used for cultivar identification. Pradeep and Jambhale (2002) reported that the activity of polyphenol oxidase was significantly higher in resistant genotypes Goli and Villaiti as compared to Darakhi-1 and susceptible types Dandan Chhuhara and Kadaka. Higher amount of total phenols were noticed in unripe fruits of resistant genotypes of Ber.

Strobilurins

Strobilurins are new class of fungicidal compounds (Margot *et al.,* 1998) that were isolated from inconspicuous woodland basidiomycetes. Strobilurin A produced by *Strobilurus tenacellus* (Pers. ex Fr.) Sing which occurs naturally in decaying pine cones of *Pinus sylvestris* L. in Europe (Ypema and Gold, 1999).

The mode of action of strobilurin is inhibition of the mitochondrial respiration in fungi, which is achieved by the prevention of electron transfer between cytochrome b and cytochrome c (Becker *et al.,* 1981).

Azoxystrobin

Among the newer fungicides, azoxystrobin is the only fungicide currently available to provide effective control of downy mildew and powdery mildew, which are the two most important fungal diseases of grapevine (Baldwin *et al.,* 1996; Wilcox *et al*., 1999)

Quinone outside Inhibitors (QoI) fungicides improves the assimilation of nitrogen into plants, inhibit ethylene biosynthesis and consequently, delay senescence of fruits (Dunne, 2002). Azoxystrobin, a new class of substance is included in the QoI fungicides group (Susana *et al*., 2005). The first QoI-fungicide commercialized in the world during 1996 was

azoxystrobin (Wicks and Hitch, 2002). Azoxystrobin was created by constructing a bridging phenyl ring between the side chain and the enol ether ester toxophore. The side chain was modified to enhance photostability and increase systemicity in the plant

(Ypema and Gold, 1999). The presence of a particular combination of heterocyclic and aromatic rings in the side chain provided high activity against fungal plant pathogens and an acceptable balance between phytotoxicity and systemic properties (Baldwin *et al.*, 1996)

Azoxystrobin demonstrated both translaminar movement and some post infection activity against downy mildew (Wong and Wilcox, 2001) and powdery mildew (Wong and Wilcox, 2002) in grapes.

Bioefficacy of azoxystrobin

Gupta and Amitkumar (2008) found that systemic fungicides persisted for longer period and provided good control of the diseases. Post symptom antisporulant activity of systemic fungicides like azoxystrobin, from strobilurin group further elaborates their role in combating the ravages of the diseases even after its appearance. Matheron and Porchas (2000) reported that azoxystrobin gives 90 per cent reduction of the mycelial growth of *Phytopthora capsici* (Leonian) Sare., *P. citrophthora* Leonian, *P. parasitica* Dastur. and *P. nicotianae* Breda de Haan. @ 3000 µg ml^{-1}.

Azoxystrobin provided cent per cent control of downy mildew in grapes, when applied 1 to 5 days before inoculation and 85 per cent mean reduction of resporulation from diseased tissue, when applied 6 days after inoculation (Wong and Wicox, 2001). Wicks and Hitch (2002) evaluated the efficacy of azoxystrobin for the control of powdery mildew and downy mildew of grapes and found that azoxystrobin @ 0.5g l^{-1} was more effective than Flint and Thiovit. Azoxystrobin applied as protectant provided cent per cent control of *U. necator* at the recommended rate of 250 µg ai ml^{-1} (Wong and Wilcox, 2002).

Willis and Duvenhage (2003) reported that azoxystrobin 250 SC @ 4 ml l^{-1}, reduced the post harvest anthracnose of avocado (3.58 %) compared to control (22.92 %). Hoffman and Wilcox (2003) found that azoxystrobin (128 mg l^{-1}) provided 78 per cent control of lesion formation and erratic control of pycnidium formation in grapes, when applied two days after the inoculation

of conidia (2 X 10^4 ml^{-1}) of the pathogen *Guignardia bidwellii* (Ell.) Viala & Ravaz. causing black rot in grapes.

Persistence of azoxystrobin

Residues of azoxystrobin (Amistar 25 SC) in the samles of grapes (fruits) initially were more in samples collected on 0^{th} day a*fter spraying in all the concentrations *viz.*, 125, 150, 200, 250, 300, 400 and 500 g a.i/ha recorded 1.20,1.60,1.97,2.38,2.91,3.76 and 5.80 in the first season, 2.13, 2.68, 4.33, 5.86, 6.95, 9.26 and 11.43 in the second season and 1.93, 2.42, 3.76, 3.76, 3.92, 4.81, 6.27 and 7.92 in the third season, respectively. Later as the time interval increased, the residues in the samples decreased and it is below detectable level from 14^{th} day after spraying (Sendhil Vel, 2003).

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CHAPTER 17

BIOLOGICAL CONTROL FOR THE MANAGEMENT OF GROUNDNUT WILT DISEASE INCITED BY *F. OXYSPORUM*

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Introduction

Wilt of groundnut is caused by *F. oxysporum*. A survey was conducted in three southern districts of Tamil Nadu for the occurrence of *Fusarium* wilt of groundnut. Groundnut plants affected with wilt exhibited greyish green discoloration and flaccidity of leaves followed by yellowing of foliage and wilting. Vascular browning of internal tissues was also noticed. In the pathogenicity tests carried out *in vitro* as well as *in vivo*, plants inoculated *F.oxysporum* produced the same symptoms as observed in the field. The findings corroborate with that of Jofee (1973) who observed bleaching of foliage, drying of canopy with vascular browning of tap roots in wilt of groundnut caused by *F.oxysporum.* In cotton infection caused by *F. oxysporum*f.sp.*vasinfectum* lead to loss of leaf turgidity, leaf yellowing and withering. Wilting was either partial or complete. Tap roots were stunted with browning and blackening of vascular tissues (Prakasam *al.,* 1993). In gingelly symptoms of *Fusarium* wilt include partial or total wilting of plants at flowering and podding,with a purple band extending from the base upwards. When the main stem or primary branches were split browning or blackening of internal tissue was noticed (Correll, 2005).

Morphological features of F. *oxysporum*

Morphological characters are important criteria in identifying *Fusarium* spp. In the present study, the isolates of *F. oxysporum* varied in growth and morphological features. Of the five isolates, I_1 was fast in growth. The colony colour of the isolates varied from white to pinkish yellow. The isolates were dense, sparse and flat in nature. All the isolates produced macro conidia, micro conidia and chlamydospore. Macro conidia were 3 to 5 septate while the micro conidia were single celled and oval in shape. In cymbidium,

F. oxysporum produced micro conidia that were oval to cylindrical in shape. The fungus produced large number of fusoid, falcate macro conidia with 3 to 5 septa,

Variability studies using molecular markers

Molecular tools have been used to characterize the diversity among pathogenic isolates of *F.oxysporum.* Molecular markers of RAPD have been used extensively as genetic markers in different fungal populations. In the present study, the genetic similarity among the *F. oxysporum* collected from five locations was between 27 % to 55 %. Forty isolates of *F.o.*f.sp.*vasinfectum* were characterized by RAPD and AFLP markers and cluster analysis showed two groups of isolates. Both techniques generated specific genomic patterns, which differentiated closely related races. Unique fingerprint profiles generated by the RAPD and AFLP techniques can be exploited for race identification but additional pathogenicity testing on differential cultivars is needed to ascertain their precise determination. . High diversity among the 20 isolates of *F. o.* f.sp. *ciceri was* was observed by RAPD technique. Though some of the isolates appeared similar in morphological and virulence patterns. RAPD finger printing could differentiated them.

Culture media suited for the growth of *F. oxysporum*

The results from the present study showed that Richard's agar as well as broth promoted the maximum mycelial growth of *F. oxysporum* followed by carrot dextrose agar.

Fig 1. F. oxysporum on different solid media

Fig 2.F.oxysporum on different Liquid mec

Carbon, nitrogen sources and the growth of *F.oxysporum*

Among the carbon sources tested, glucose was found to promote fast growth of *F.oxysporum* in solid media and yielded more mycelial weight in liquid culture.

In the present study when the nitrogen sources were compared, ammonium nitrate stood first followed by potassium nitrate for culturing *F.oxysporum*. Both were equally efficient in promoting the fast growth of the fungus on solid medium and yielding more mycelium in liquid broth. These results are similar to the findings of. Peptone followed by potassium nitrate was the best source of nitrogen for the growth of *F. oxysporum*

Fig 3.Growth of F.oxysporum on carbon sources

Fig 4.Growth of F.oxysporum on nitrogen sources

pH levels and the growth of *F. oxysporum*

In the present study, the maximum mycelial growth of *F.oxysporum* was observed at pH 6.5 followed by pH 7.0 and the least growth was at pH 8.0.

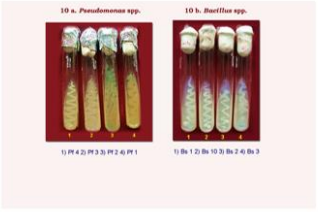

Fig 5. Isolation of bacterial antagonist from different rhizosphere soil

Fig 6. Growth of F.oxysporum on different pH

Bacterial antagonists and *F. oxysporum*

Though all the four isolates of *P. fluorescens* retarded the mycelial growth of *F. oxysporum*, Pf_1 was found to be more effective. The results agree with those of

Fungal antagonists and *F. oxysporum*

Organic amendments and *F. oxysporum*

In the present study, the extract of mahua cake was found exhibit higher inhibitory effect on *F.oxysporum.* The lowest mycelial growth of *F. solani* was observed in mahua cake incorporated medium. The aqueous

extract of neem cake inhibited the growth of *F. o.* f. sp. *ciceri*. Neem cake has been found to completely control *F. solani* infection in 40 day-old soybean plants /The extracts of neem cake at 10 per cent were found to be effective against *F. solani* and caused 80.4 per cent mycelial growth inhibition followed by mahua cake (75.1%). The neem cake extract was highly inhibitory to *F. o.* f. sp. *lycopersici*. (Ali, 1997). Extract of neem cake showed excellent inhibitory effect against the chilli wilt pathogen *F. solani*. The radial growth of *F. o.* f. sp. *psidii* was significantly less in neem leaf extract incorporated medium

Fungicides and *F. oxysporum*

In the present study, carbendazim, benomyl and Saff at 0.05 per cent completely inhibited the growth of *F. oxysproum***.**

Mode of action

Antibiosis

In the present investigation, antibiotic of Pf_1 exhibited more inhibitory effect on *F. oxysporum* followed by Pf_2, BS_{10} and BS_2. Several strains of *Pseudomonas* spp. and *Bacillus* spp. produce wide array of antibiotics which include lacton, 2- 4 diacetylphloroglucinol (2-4 DAPG), HCN, oligomycin, oomycin A, phenazine, pyrolnitrin, pyocyanin, surfactin and several uncharacterized molecules

Florescent pseudomonads in the plant rhizosphere have been found to improve the plant growth and suppression of plant disease by the production of antibiotics, siderophores, hydrolytic enzymes and HCN .the naturally occurring *fluorescent pseudomonads* produced the antibiotic, 2-4 DAPG. *Bacillus* spp produced different inhibitory agents which have been categorized in peptide derivative family (. Bacilysocin, a novel and broad spectrum phospholipid antibiotic was purified from *B. subtilis* strain 168 The *P. fluorescens* produced secondary metabolites such as siderophores, HCN and protease which showed antagonistic activity against *Fusarium* spp.

Volatiles and disease suppression

In the present investigation, the inhibitory effect of volatiles released by Tv_1 was more pronounced than that of Pf_1 and BS_{10}. Diffusible volatile compounds produced by *T. viride* and *T. harzianum* inhibited the germination and mycelial growth of *F. oxysporum.*

The volatile metabolite furanone produced by *P. aureofaciens* showed antifungal activity against *F. solani*, *F. oxysporum*, *P. ultimum* and

Thielaviopsis bassicola. The involvement of volatile and nonvolatile antibiotic compounds released by *Trichoderma* spp. against the sugar beet root rot pathogen *S. rolfsii*. Bacterial strains of *P. fluorescens* inhibited the mycelial growth of *F. o. f.* sp. *dianthi* by production of volatile metabolites under laboratory condition. Retarded radial growth of *F. oxysporum* infecting groundnut was due to the volatile and non- volatile metabolites produced by *Trichoderma* spp.

The isolate Bs_{10} was found to release volatiles that was inhibitory to *F. oxysporum. Bacillus* spp are ubiquitous in the environment and found associated with antifungal activity by producing volatile compounds as well as non-volatile substances.

HCN and disease suppression

Production of HCN by certain strains of fluorescent pseudomonads has been involved in the suppression of soil-borne pathogens. In our study, production of HCN was very strong in Pf_1 than in Pf_2. Role of HCN in disease suppression has been demonstrated by several scientists in various crops HCN is the common secondary metabolite produced by rhizosphere pseudomonads the HCN production of several strains of *P. fluorescens* and their efficacy in controlling root rot of groundnut caused by *M. phaseolina. Pseudomonads* releasing HCN were reported in the rhizosphere of tobacco in soils suppressive to *T. bassicola*, causal agent of black root rot of tobacco

Siderophores and disease suppression

Pseudomonads generally produce fluorescent, yellow-green, water soluble siderophores. The siderophores are either pyoveridins or pseudobactins. Production of the siderophores has been linked to the disease suppressive potential of certain florescent pseudomonads. In the present study, the strain Pf_1 produced more quantity of siderophore than Pf_2 and the siderophore was hydroxymate type. The fungal strain Tv_1 also produced siderophore.

Siderophore of *Pseudomonas* spp inhibited the chlamydospore germination of *F. s.* f. sp. *Lini*, *F. o.* f.sp. *cucurbitae* and *F. o*. f. sp. *cucumerinum*. The production of fluorescent siderophore by P. *fluorescens* which was attributed to its antagonistic action. Under iron deficiency, the culture filtrate of all strains of *Trichoderma* contained coprogen, coprogen B and ferricrocin as siderophore. *T. longi* and *T. pseudokoningii* produced fuigen type of siderophore. The hydroxymate type of siderophore was ferribactin produced by *P. fluorescens*. The siderophore of *P. fluorescens* GL20 inhibited spore

germination and hyphal growth of *F. solani in vitro* and reduced the disease incidence with enhanced plant growth. Siderophore of *Fluorescens* was inhibitory to the growth of *M. phaseolina in vitro*. *B. subtilis* (BSCBE4), *P. chlororaphis* (PA23) and *P. fluorescens* produced both hydroxymate and carboxylate type of siderophores. The siderophore production and antifungal activity was exhibited by 10 to 12.7 per cent of pseudomonas isolates.

Salicylic acid

The PGPR strains are capable of producing SA and are responsible for the induction of ISR in plants. In the current study, SA production was observed to be more in Pf_1 while it was less in Bs_{10}. Role of SA producing *P. aeruginosa* in disease suppression. Inoculation of roots of chickpea with *P. fluorescens* strain H92 (or) with synthetic 0-acetyl salicylic acid induced systemic resistance against the charcoal rot fungus, *M. phasiolina*

The production of SA was maximum in *P. fluorescens* strain Pf_1 followed by ALR-7 and Pf MDU 2 isolates. There was a significant relationship between inhibitory activity of *P. fluorescens* strains *in vitro* and their level of SA production. SA production has been observed for several bacterial strains and exogenously applied SA induces resistance in plant species.

Induced systemic resistance

P. fluorescens could act as strong elicitor of plant defense reaction Induced resistance by *P. fluorescens* is associated with accumulation of pathogenesis related proteins. Application of fluorescent pseudomonads strengthen the cell wall structures resulting in restriction of pathogen invasion in plant The increased level of PO and PAL in roots treated with *P. fluorecens* against Fusarium wilt of banana. In our investigation enhanced activities of defense related enzymes were observed in groundnut plants in response to the application of biocontrol agents against *F. oxysporum* indicating the induction of systemic resistance.

Phenylalanine ammonia lyase

The present study revealed that all the biocontrol agents induced the plants to synthesize more of PAL. The maximum PAL activity was observed on sixth day in plants treated with Tv_1 and BS_{10} when challenged with the pathogen. The product of PAL activity in transcinnamic acid which is an immediate precursor for the biosynthesis of SA, a signal molecule in SAR. PAL activity could be induced during plant-pathogen interactions. Induction of PAL by florescent pseudomonads was reported in cucumber against *P.*

aphanidermatum. When turmeric plants were sprayed with *P. fluorescens*, PAL activity got enhanced

Peroxidase

Peroxidase is a key enzyme in the biosynthesis of lignin (Bruce and West, 1989). Increased activity of PO has been elicited by fluorescent pseudomonads in rice sheath blight (Nandakumar *et al*., 2001; Radjacommareet *et al*.,2002), black gram root rot (Karthikeyan *et al*., 2003) and groundnut late leaf spot (Meena *et al*.,2000) against plant pathogens. -In the present study, groundnut plants treated with the combination of $Tv_1$and BS10 when challenged with the pathogen showed increased activity of PO.

Accumulation of PO has been correlated with ISR in several crops (Ramamoorthy and Samiyappan, 2001). Isolates of *Pseudomonas* systemically induced resistance against Fusarium wilt of chickpea and suppressed the disease by 34.45 per cent when compared to control (Saikia *et al*., 2005). Mandal (2009) reported that exogenous application of SA could induce resistance against *F. o*. f. sp. *lycopersici* in tomato.

Polyphenoloxidase

PPO is a copper containing enzyme which usually accumulates on wounding in plants. Induction of PPO activity has been correlated with a resistance response Expression of new PPO isoform was observed in *P. fluorescens* Pf_1 treated tomato plants challenged with *F. o*. f. sp. *Lycopersici* induction of PPO enzymes in plant growth promoting bacteria treated banana plants. The accelerated PPO activity in chilli plants treated with *P. fluorescens* when challenge inoculated with *C. capsici*. The application of *P. fluorescens* and its combination with *B. subtilis* significantly increased PPO against *P.aphanidermatum* and *M. phaseolina* respectively.

Plant growth promotion

In the present investigation, application of biocontrol agents increased the shoot and root lengths of groundnut in sand tray method. The Plant growth enhancement was more in Tv_1, Pf_1 and Bs10 combination followed by Tv_1 and Bs10. Similar results have been documented in many crops by earlier workers.

Application of *B. subtilis* F2B24 increased the growth and yield in peanut use of *B. subtilis* Af_1promoted seed germination and biomass of groundnut and pigeon pea even at high pathogen pressure. The P. *fluorescens* (Pf_1) and *B. subtilis* increased the seed germination and seedling vigour of chillies. Similarly, promotion of plant growth by *P. fluorescens*, *Bacillus* spp. and *Trichoderma* spp. has been documented by various workers.

Management of *Fusarium* wilt using biocontrol agents, organic amendments and fungicides under glasshouse condition

In the present study, carbendazim (0.1%) was found to be highly effective in controlling Fusarium wilt of groundnut followed by combined application of biocontrol agents with organic amendment. the effectiveness of carbendazim and thiram against safflower wilt pathogen, *F. o.* f. sp. *cathami*. The carbendazim when used as basal compound against *Fusarium* sp. on cotton. Sugha *et al*. (1995) reported that carbendazim was very effective in reducing the Fusarium wilt disease. The chemical treatment with benomyl and carbendazim was proved to be the most effective against *F. o*. f. sp. *ciceri.* In our investigation application of Tv1, Pf1 and Bs10 with mahua cake reduced the disease incidence up to 16.09 per cent. The application of biocontrol agents *viz*., *T. harzianum*, *B. subtilis*, *P. fluorescens* reduced the Fusarium wilt incidence in safflower both under greenhouse and field condition. The combined application of two strains of *P.flourescens* with *T.harzianum* caused only 6.8 per cent incidence of Fusarium wilt in onion which indicated 85.2 per cent disease reduction. The maximum control of (44.4%) of wilt of tomato was observed in *T. harzianum* treated plants when compared to control.

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CHAPTER 18

ENTOMOPATHOGENIC NEMATODES: BIOLOGY AND APPLICATIONS

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Abstract

Entomopathogenic nematodes (EPNs) belonging to the families Heterorhabditidae (Genus: *Heterorhabditis*) and Steinernematidae (Genus: *Steinernema*) are mutualistically associated with bacteria in the family Enterobacteriaceae (*Photorhabdus* spp. for *Heterorhabditis* and *Xenorhabdus* spp. for *Steinernema*). At present, there are 100 *Steinernema* and 17 *Heterorhabditis* species and 20 *Xenorhabdus* and 4 *Photorhabdus* species. In general, each EPN species has its own bacterial species, but a given bacterial species may be associated with more than one EPN species. The EPNs' natural habitat is the soil where the nematode-bacterium complex infects many different insect species killing them within 48 hrs. EPNs have been isolated from many different islands and from all continents except Antarctica. Because EPNs and their associated bacteria are safe to humans, other vertebrates and plants can effectively kill soil insect pests in a short time, serve as an alternative to chemical pesticides, are easily massed produced *in vivo* and *in vitro* and do not require registration in many countries, a number of EPN species have been produced commercially to target soil and plant-boring pests in high value crops. Moreover, the associated bacteria produce antibiotics and other compounds that have potential to be used against human, veterinary and plant pathogens.

Key words: EPN, *Steinernema*, *Heterorhabditis*, Biological control, non-chemical.

1. Introduction

Nematodes are non-segmented, elongated roundworms that are colorless, without appendages and usually microscopic. There are non-beneficial and beneficial nematodes. Non-beneficial nematodes are also called "plant parasitic nematodes" and cause damage to crops and beneficial nematodes attack soil borne insect pests, yet are not harmful to humans, animals, plants, or earthworms, and can therefore be used as biological control organisms [1]. Beneficial nematodes that cause disease within an insect are referred to as "Entomopathogenic" and have the ability to kill insects. Although nearly 40 nematode families are associated with insects, very few nematodes can cause host mortality belongs to two families, Steinernematidae and Heterorhabditidae are widely available for biological control. EPNs have been well known since 1923, when Steiner [2] identified the species *Aplectana kraussei*. Later, [3] identified a nematode infecting grubs of the Japanese beetle, *Popillia japonica* at the Tavistock Golf Course near Haddonfield, New Jersey, USA. This nematode was described by Steiner as *Neoaplectana* (=*Steinernema*) *glaseri* (Rhyabditida: Steinernematidae) from Belgium as a natural pathogen of *Hoplia philanthus* (Coleoptera: Scarabaeidae). Later, his colleagues propagated sufficient amounts of the species for field trials. A new species of entomopathogenic nematode, *Heterorhabditis bacteriophora,* was described in 1975, as a new species as well as a member of new genus and family (Heterorhabditidae) of Rhabditida [4]. The family is very similar to the family Steinernematidae. In the last three decades, many EPNs have been carried out in different habitats all over the world, revealing hundreds of new isolates and many new species [5]. Currently, over 80 species of *Steinernema* and 20 species of *Heterorhabditis* have been described (NCBI, 2015).

2. Biology of EPNs

Three unique attributes of *Steinernema* and *Heterorhabditis* nematodes make them interesting model system for application in biological control.

1. First, they form a complex nematode-bacterium mutualistic symbiosis. The bacteria are carried in the body of nematodes and released into hosts [6].
2. Second, they are insect pathogens with a very broad host spectrum that includes the majority of insect orders.

3. Third, they can be cultured either *in vivo* or *in vitro* on a large scale. Even though the two groups of nematodes can infect, kill and emerge as a new generation from insects in a similar way, their life cycles are different.

The life cycle of the entomopathogenic nematodes (EPNs) *Steinernema* and *Heterorhabditis* is subdivided into the so-called larvae stages. The infective juvenile (IJ)/ or (dauer) represents the only stage of the nematode outside of their insect host. At this stage, the nematode is a non-feeding and soil-dwelling larvae, encased in a double cuticle with closed mouth and anus and able to survive for long-terms in the soil.

3. Foraging strategy

1. Cruiser: IJs of the family Heterorhabditiae use cruiser strategy to search actively in the soil for suitable insect larvae.

2. Ambusher: Nematodes of the family Steinernematidae adopted the ambusher strategy, waiting passively near the soil surface for prey to cross their way. After an insect is sensed, the nematode sheds its outer cuticle to uncover mouth and anus, enters the insect through natural openings like anus, mouth and spiracles and migrates to the insect blood cavity [6].

In comparison to *Steinernema*, *Heterorhabditis* is able to penetrate directly through the thin intersegmental areas of the insect integument by using a dorsal tooth. Both of steinernematid and heterorhabditid nematodes are associated with the symbiotic bacteria *Photorhabdus* and *Xenorhabdus* [7]. The bacteria are gram-negative, with facultative anaerobic rods in the family Enterobacteriaceae are found within the intestine of the infective juvenile (IJ) nematode. An IJ carries between 0 and 2000 cells of its symbiont bacterium in the anterior part of the intestine. *Xenorhabdus* occurs naturally in a special intestinal vesicle of *Steinernema* IJs, while *Photorhabdus* is distributed in the foregut and midgut of *Heterorhabditis* IJs [8].

The relationship between the nematode and the symbiotic bacterium is a type of symbiosis, where both benefit from the association. The nematode provides protected shelter for the symbiotic bacteria and carries the bacteria into the host. After entering the host, the nematode penetrates through the gut wall and regurgitates symbiotic bacteria into the insect hemocoel. Nematode and bacteria overcome the insect immune system and the host insect is killed within 48 hours post infection [9]. The bacteria break

down the host tissues and provide food sources for the nematode, which feeds and multiplies on bacterial cells and degrading host tissues. During the process, the bacteria provide the nematode and themselves a protected niche by producing antibiotics that suppress the competition from other microorganisms [10]. Due to the different symbiotic bacteria associated with EPN, heterorhabditid nematodes turn the host cadaver red, purple, orange, yellow, brown or sometimes green, whereas steinernematid nematodes turn the insect cadaver tan, ochre, gray or dark gray.

The first stage after entering the insect is the so-called recovery phase (J3). Triggered by a unknown food signal, the nematodes exit the infective stage in a developmental step that is known as recovery and transform into the fourth stage (J4), causing a toxicogenesis or septicemia by releasing an immunosuppressive factor, that inhibits antimicrobial peptides, produced by the insect. J4 stages nematodes develop into egg lying female or male adults in the insect cadaver and hereby run through four juvenile stages (J1 - J4) and the adult stage has up to three generations [10]. After reproduction and depletion of all nutrients, a high nematode population density triggers the nematode development into IJs again. In the case of *Steinernema*, IJs become colonized by bacteria *via* one or two founder bacterial cells. Finally, dependent on the size of the insect prey, up to several hundred thousand individuals emerge from the empty carcass. The life cycle of *Heterorhabditis* is similar to that of *Steinernematids* except for the fact that the IJs always develop into self-reproducing hermaphrodites [11]. An offspring of the first generation hermaphrodites can either develop into amphimictic adults or into automictic hermaphrodite, both can occur simultaneously. The development into amphimictic adults is induced by favourable nutritional conditions, whereas the development of hermaphrodites is induced by low concentrations of nutrient. The life cycle is completed in a few days and thousands of new IJs emerge, searching for new hosts. The cycle from entry of IJs into a host until emergence of new IJs is dependent on temperature and varies for different species and strains. Recently, other nematode species have been shown to use pathogenic bacteria to parasitize insect hosts. Two *Oscheius* (=*Heterorhabditoides*) species, *O. chongmingensis* and *O. carolinensis*, and *Caenorhabditis briggsae* have been identified as potential insect pathogens by baiting soil for nematodes using insect larvae as prey, a common approach used for finding

EPNs. All of these have been found to associate with insect pathogenic bacteria of the genus *Serratia*, while *O. carolinensis* may have additional associates [12]. *O. chongmingensis* and *C. briggsae* require their bacterial partners to cause host death, to grow and reproduce within killed insects, and emerging dauer juveniles are associated with the vectored pathogen

4. Production and formulation

EPNs are currently mass produced by different methods either *in vivo* or *in vitro* [13].

In vivo: *In vivo* production is considered the most appropriate technology for growers cooperatives and for developing countries, where labor is less expensive [14]. In addition, it is a simple process of culturing specific EPNs in live insect hosts, which requires less capital and technical expertise. *In vivo* production system is based on the White trap, which take advantage of the IJ's natural migration away from host cadaver upon emergence. The most common insect host used for *in vivo* production is the last instar of the greater wax moth *Galleria melonella* (L.) (Lepidoptera: Pyralidae). Producing the greater wax moth in mass has many complications, including the production of cocoons and the extreme fragility of nematode infected larvae. The Yellow mealworm, *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae), is an alternative host for *in vivo* nematode production, which does not produce cocoons and retains structural integrity, while infected by nematodes. Mealworms have the additional advantage of being produced commercially in large quantities in many countries.

Nematodes produced *in vivo* using these technologies have been proven effective against the citrus weevil (*Diaprepes abbreviatus*) and the small hive beetle (*Aethina tumida*) and may be effective against other important insect pests. Methods to produce mealworms in mass do not require the use of sophisticated technology and can be implemented in less industrialized countries. The most important requirement for successful and economically reasonable usage of EPNs in crop protection is large scale production at low cost within a short process time [15]. This can only be achieved under well-defined liquid culture conditions and successful management of nematode population dynamics. Nowadays, EPNs are produced for commercial purposes by several companies in large liquid

fermentation tanks which range from 50,000 up to 100,000 liter fermentation system.

In vitro: *In vitro* culturing of EPNs is based on introducing nematodes to a pure culture of their symbiont in a nutritive medium. A liquid medium is mixed with foam, autoclaved and then inoculated with bacteria, followed by the nematodes. Nematodes are then harvested within 2-5 weeks [16] by placing the foam onto sieves immersed in water. Media include various ingredients including peptone, yeast extract, eggs, soy flour, and lard. Nematodes can be stored and formulated in different ways including the use of polyurethane sponge, water-dispersible granules, vermiculite, alginate gels and baits. Formulated EPNs can be stored for 2 to 7 months depending on the nematode species and storage media and conditions. Unlike other microbial control agents (fungi, bacteria and virus) EPNs do not have a fully dormant resting stage and they will use their limited energy during storage. The quality of the nematode product can be determined by nematode virulence and viability assays, age and the ratio of viable to non-viable nematodes [17].

5. Application methods

EPNs can be applied with nearly all agronomic or horticultural ground equipments including pressurized sprayers, mist blowers and electrostatic sprayers or as aerial sprays [18] The application equipment used depends on the cropping system and in case there are a variety of handling considerations including volume, agitation, nozzle type, pressure and recycling time, system of environmental conditions and spray distribution pattern [19]. It is important to ensure adequate agitation during application.

For small plot applications, hand held equipment or back-pack sprayers may be appropriate. When nematodes are applied to larger plots, a suitable spraying apparatus, such as a boom sprayer, should be considered. Applicators could also be using other methods, such as through microjet irrigation systems, subsurface injection or baits [20]. Various formulations for entomopathogenic nematodes may be used for applying EPNs in aqueous suspension, including activated charcoal, alginate and polyacrylamide gels, clay, peat, polyurethane sponge, vermiculite, and water dispersible granules (WDG).

Enhanced efficacy in EPN applications can be facilitated through improved formulation. Substantial progress has been made in recent years in

developing EPN formulations, particularly for aboveground applications, such as mixing EPNs with a surfactant and polymer [21]. Improved efficacy may also be achieved by relying on leaf flooding with the addition of surfactants to increase leaf coverage [22]. Additionally, *S. carpocapsae* applications for control of the lesser peach tree borer, *Synanthedon pictipes*, were greatly improved by a follow-up application of a sprayable gel, the gel is commonly used for protecting structures from fire [23]. *S. carpocapsae* caused high levels of suppression (98% efficacy in a preventative treatment) in case of the red palm weevil, *Rhynchophorus ferrugineus*, when applied in a chitosan formulation.

In the same context, efficacy of EPN applications can also be enhanced through improved application equipment or approaches. Despite well-established procedures, equipment used for entomopathogen application can be improved further, e.g. optimizing spray systems (e.g. nozzles, pumps, spray distribution) for enhancing pathogen survival and dispersion [24]. Bait formulations can enhance EPN persistence and reduce the quantity of microbial agents required per unit area [25]; though limited thus far, conceivably, baits can be developed further for wide applications. Another novel application approach that has gained attention is delivery of EPNs in their infected host cadavers. Another most stricking observation is the fact that application of EPNs in capsules, prepared from several compounds, including polysaccharide extracted from the algae, *Laminaria* spp. [26] are easy to apply in the field. From these capsules entomopathogenic nematodes can easily break through, and successfully infect insect pests, such *as Diabrotica virgifera*. In addition, these nematode-filled capsules can attract insect pests in the field if they are coated with insect food stimulant or attractants.

Application of cadavers may be facilitated through formulations that have been developed to protect cadavers from rupture and improve handling process [27] and development of mechanized equipment for field distribution [28]. The period of six to ten days between infection and application on soil of *Galleria mellonella* cadavers resulted in higher emergence of IJs and was thus recommended when using the cadaver application approach. Lately, [29] stated that nematodes applied in host cadavers were effective and persistent when added to bags of potting media for subsequent distribution to target pest sites.

6. Use of EPNs in biocontrol

6.1. Biological control

There are three strategies of biological control: classical, augmentative and conservation control [30].

6.1.1 Classical control: involves importing and releasing the parasitoid or predator of an exotic pest that has become established in a new region. The parasitoid or predator is expected also to establish itself in its new environment, so that no further releases are necessary.

6.1.2. Augmentative control: divided into two sub categories:

1. **Inundative release:** the application of large numbers of the control organism against a pest.
2. **Inoculative release (seasonal):** in which the control organism is released once in a season and is expected to produce progeny that will continue to control the pest throughout the growing season.

6.1.3. Conservation biocontrol: refers to a whole set of measures that can be taken to favour the population buildup of indigenous natural enemies of (native) pests (e.g. creating refuges and providing alternative food for natural enemies).

The use of EPNs in biocontrol has a long history. Early uses going back to the 1930s were geared towards classical biological control, as in the case of the introduction of *S. glaseri* to control the Japanese beetle *Popilla japonica* in the USA. EPNs re-emerged as potential biocontrol agents in the 1960s and 70s, with research mainly focusing on *Neoaplectana* =(*Steinernema) carpocapsae* [31]. Several EPN species are now produced commercially and available in a formulation suitable for short-term storage. Since IJs can now be produced relatively cheap in large numbers, the preferred method of application is inundative, i.e. short term application of large numbers of nematodes to create a direct impact on the pest population [32].

The vast majority of applied research has focused on their potential as inundatively applied to augment biological control agents [33] these can be considered as good candidates for integrated pest management and sustainable agriculture due to a variety of attributes. In addition, some species can recycle and persist in the environment; they may have direct and/or indirect effects on populations of plant parasitic nematodes and plant

pathogens; can play an indirect role in improving soil quality; and are compatible with a wide range of chemical and biological pesticides used in IPM programs. Table 1 show current use of *Steinernema* and *Heterorhabditis* nematodes, as biological control organisms [34] and modified by [35].

Crops/ Targets	**Insect pests**		**Nematodes***
	Common name	**Scientific name**	
Artichokes	Artichoke plume moth	*Platyptilia carduidactyla*	Sc
Vegetables	Armyworm	Lepidoptera: Noctuidae	Sc, Sf, Sr
Ornamentals	Banana moth	*Opogona sachari*	Hb, Sc
Bananas	Banana root borer	*Cosmopolites sordidus*	Sc, Sf, Sg
Turf	Billbug	*Sphenophorus* spp.	Hb,Sc
Turf, vegetables	Black cutworm	*Agrotis ipsilon*	Sc
Canola	Black cutworm	*Agrotis ipsilon*	Sc, Hb
Berries, ornamentals	Black vine weevil	*Otiorhynchus sulcatus*	Hb, Hd, Hm,
Fruit trees, ornamentals	Borer	*Synanthedon* spp. and other *Sesiids*	Hb, Sc, Sf
Home yard, turf	Cat flea	*Ctenocephalides felis*	Sc
Citrus, ornamentals	Citrus root weevil	*Pachnaeus* spp.	Sr, Hb
Pome fruit	Codling moth	*Cydia pomonella*	Sc, Sf
Canola	Diamondback moth	*Plutella xylostella*	Sc, Hb
Vegetables	Corn earworm	*Helicoverpa zea*	Sc, Sf, Sr
Vegetables	Corn rootworm	*Diabrotica* spp.	Hb, Sc
Cranberries	Cranberry girdler	*Chrysoteuchia* topiaria	Sc
Turf	Crane fly	Diptera: Tipulidae	Sc
Citrus, ornamentals	Diaprepes root weevil	*Diaprepes abbreviatus*	Hb, Sr
Mushrooms	Fungus gnat	Diptera: Sciaridae	Sf, Hb

Grapes	Grape root borer	*Vitacea polistiformis*	Hz, Hb
Iris	Iris borer	*Macronoctua onusta*	Hb, Sc
Forest plantings	Large pine weevil	*Hylobius albietis*	Hd, Sc
Vegetables, ornamentals	Leaf miner	*Liriomyza* spp. (Diptera: Agromyzidae)	Sc, Sf
Turf	Mole cricket	*Scapteriscus* spp.	Sc, Sr, Sscap
Nut and fruit trees	Navel orangeworm	*Amyelois transitella*	Sc
Fruit trees	Plum curculio	*Conotrachelus nenuphar*	Sr
Stone fruit orchards	Flat-headed root borer	*Capnodis tenebrionis*	Sc, Sf
Date palm	Red palm weevil	*Rhynchophorus ferrugineus*	Sc
Turf, ornamentals	Scarab grub	Coleoptera: Scarabaeidae	Hb, Sc, Sg, Ss,Hz
Ornamentals.	Shore fly	*Scatella* spp	Sc, Sf
Berries	Strawberry root weevil	*Otiorhynchus ovatus*	Hm
Strawberry	Root weevil	*Otiorhynchus ovatus*	Hm
Bee hives	Small hive beetle	*Aethina tumida*	Hi, Sr
Sweet potato	Sweetpotato weevil	*Cylas formicarius*	Hb, Sc, Sf
Termites hills	Subterranean termites	*Psammotermes hypostoma*	Sc, Hb

** H. downesi*, Hi = *H. indica*, Hm = *H. marelata*, Hmeg = *H. megidis*, Hz = *H. zealandica*, Sc = *Steinernema carpocapsae*, Sf = *S. feltiae*, Sg = *S. glaseri*, Sk = *S. kushidai*, Sr = *S. riobrave*, Ss cap = *S. scapterisci*, Ss = *S. scarabaei*.

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CHAPTER – 19

SAFE USE OF CHEMICAL CONTROLS IN INTEGRATED DISEASE MANAGEMENT

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Abstract
Chemical controls are a critical component of Integrated Disease Management (IDM), providing targeted interventions to manage plant and crop diseases effectively. This chapter addresses the principles and practices for the safe and responsible use of chemical controls within the IDM framework. It highlights the importance of integrating chemical controls with other management strategies to reduce the risk of resistance development, environmental impact, and human health hazards. The chapter reviews key concepts such as dosage, application methods, safety precautions, and regulatory considerations. Case studies and best practices are discussed to illustrate effective chemical control strategies in various agricultural contexts.

1. Introduction

1.1 Definition of Integrated Disease Management (IDM)

Integrated Disease Management (IDM) is a holistic and sustainable approach to managing plant diseases by combining multiple control strategies—cultural, biological, mechanical, botanical, and chemical—in a coordinated manner. The goal of IDM is to reduce the economic damage caused by diseases while minimizing negative environmental and health impacts. Rather than relying solely on any one method, IDM emphasizes the integration of compatible techniques based on ecological principles and scientific knowledge.

1.2 Role of Chemical Controls in IDM

Chemical control, involving the use of fungicides, bactericides, and other pesticides, remains an important component of IDM, particularly in high-

value or high-risk crops. However, in IDM, chemical use is not routine or preventive but judicious, targeted, and need-based**,** guided by disease monitoring, forecasting, and economic threshold levels. When used correctly, chemical control can provide rapid and effective disease suppression, help prevent crop losses, and serve as a complement to other management practices.

The goal is not elimination of chemical use, but optimization—maximizing effectiveness while minimizing negative consequences such as resistance development, pesticide residues, environmental contamination, and harm to non-target organisms.

1.3 Objectives of the Safe Use of Chemical Control in IDM

- Emphasize the importance of responsible and informed pesticide use within IDM frameworks.
- Outline best practices for the safe handling, storage, application, and disposal of chemical products.
- Discuss the risks of misuse**,** including resistance development, toxicity, and environmental harm.
- Promote training, personal protective equipment (PPE) use, and adherence **to** label instructions and legal regulations**.**

2. Principles of Chemical Controls

2.1. Mechanisms of Action

Chemical controls work by targeting specific physiological or biochemical processes in pathogens. The mechanism of action determines how the chemical interferes with the pathogen's survival, reproduction, or infection capability.

- **Inhibition of cell wall synthesis**: Some fungicides (e.g., Echinocandins) interfere with the synthesis of fungal cell walls.
- **Disruption of cell membrane**: Certain chemicals (e.g., Polyenes) bind to cell membranes, increasing permeability and causing leakage of cellular contents.
- **Inhibition of nucleic acid synthesis**: Some bactericides or fungicides prevent DNA/RNA synthesis, inhibiting cell division and growth.
- **Inhibition of protein synthesis**: Some chemicals disrupt the ribosomal function in pathogens.

- **Respiratory inhibition**: Some fungicides (e.g., Strobilurins) block mitochondrial respiration, cutting off the energy supply.
- **Hormonal disruption**: Nematicides may affect hormonal balance or signaling pathways in nematodes, affecting their reproduction or mobility.

Understanding the mode of action is crucial for selecting appropriate chemicals, managing resistance, and designing Integrated Disease Management (IDM) strategies.

2.2. Types of Chemical Controls

Chemical disease control agents are classified based on the target pathogen group and their mode of action:

- **Fungicides**: Used to control fungal diseases.
  - *Contact fungicides*: Stay on the plant surface; prevent fungal spores from germinating (e.g., mancozeb, copper oxychloride).
  - *Systemic fungicides*: Absorbed and translocated within the plant; provide internal protection (e.g., carbendazim, triazoles).
- **Bactericides**: Used against bacterial pathogens.
  - Often copper-based compounds (e.g., copper hydroxide) or antibiotics (e.g., streptomycin).
- **Nematicides**: Target nematodes in soil or plant roots.
  - *Fumigants*: Volatile compounds that act in the vapor phase (e.g., methyl bromide).
  - *Non-fumigants*: Absorbed by plants or nematodes (e.g., carbofuran).
- **Viricides**: Rare; mostly aim to prevent virus transmission (e.g., mineral oils to prevent vector feeding).
- **Oomyceticides**: Specific to oomycete pathogens like *Phytophthora* and *Pythium* (e.g., metalaxyl).

Each chemical is chosen based on pathogen identity, crop species, environmental conditions, and compatibility with other IDM components.

2.3. Importance of Selective Use and Dosage

Selective Use:

- Use only when necessary, based on disease forecasting or economic threshold levels.

- Avoid broad-spectrum use unless essential, to preserve beneficial organisms.
- Integrate with cultural, biological, and mechanical methods in an IDM approach.

Correct Dosage:

- Using too low a dose can lead to ineffective control and promote resistance development.
- Overdosing can cause phytotoxicity, environmental pollution, and food safety concerns.
- Follow manufacturer recommendations and local agricultural extension guidelines.

Resistance Management:

- Rotate chemicals with different modes of action.
- Avoid repeated use of the same active ingredient.
- Mix or alternate with non-chemical methods.

3. Safe Application Practices

Application Methods

Different application techniques are used based on the disease, crop type, and chemical formulation:

1. **Spraying**
   - Most common method; suitable for foliar diseases.
   - Equipment: knapsack sprayers, power sprayers, tractor-mounted sprayers.
   - Ensure uniform coverage for maximum efficacy.
2. **Soil Treatment**
   - Applied to soil to control soil-borne pathogens or nematodes.
   - Methods: soil drenching, soil incorporation, fumigation.
   - Used before planting or during early crop growth stages.
3. **Seed Treatment**
   - Chemicals applied to seeds to protect against seed- and soil-borne pathogens.
   - Increases germination and early plant vigor.
4. **Trunk Injection or Painting**
   - For perennial crops (e.g., fruit trees).
   - Injecting systemic fungicides directly into the trunk or applying as a paint.

5. **Dusting or Granule Application**
    - Dry formulations applied using dusters or manually.
    - Useful in areas with limited water availability.

Timing and Frequency of Applications

Proper timing is critical to ensure the chemical reaches the pathogen when it's most vulnerable:

- **Preventive applications**: Before disease symptoms appear, especially in high-risk conditions (e.g., wet, humid weather).
- **Curative applications**: Early after symptom onset to limit spread.
- **Frequency** depends on:
    - Pathogen life cycle
    - Residual activity of the chemical
    - Environmental conditions

Tip: Follow the label instructions or local agricultural extension schedules. Avoid unnecessary repeat applications to prevent resistance.

Personal Protective Equipment (PPE)

Using PPE is essential to protect applicators from chemical exposure:

Basic PPE includes:

- Gloves (rubber or chemical-resistant)
- Face mask or respirator
- Goggles or face shield
- Long-sleeved clothing and boots
- Apron or overall if mixing chemicals

PPE should be:

- Worn during mixing, loading, and application
- Washed regularly and stored separately
- Never shared or worn casually

Note: Lack of PPE usage can result in acute poisoning, chronic health problems, or environmental contamination.

Calibration and Maintenance of Application Equipment

Accurate equipment ensures correct dosage and uniform application:

Calibration:

- Determine the spray volume per hectare.
- Adjust nozzle pressure, flow rate, and walking speed accordingly.
- Avoid over- or under-application.

Maintenance:

- Clean sprayers after each use.
- Inspect nozzles, hoses, tanks, and pressure gauges.
- Store equipment in a dry, safe place.

4. Environmental and Health Considerations

Impact on Non-Target Organisms

Pesticides can unintentionally affect organisms that are not the target of treatment, including beneficial species and wildlife:

- **Beneficial Insects**: Pollinators like bees and natural predators of pests (e.g., ladybugs, lacewings) may be harmed by broad-spectrum chemicals.
- **Soil Microorganisms**: Repeated use of certain fungicides and bactericides can disrupt soil microbial balance, affecting nutrient cycling and soil fertility.
- **Aquatic Life**: Runoff containing pesticides can contaminate nearby water bodies, harming fish, amphibians, and aquatic invertebrates.
- **Birds and Mammals**: Secondary poisoning may occur when these animals consume contaminated prey or water.

Mitigation: Use selective pesticides, avoid spraying during flowering, and implement buffer zones around water bodies.

Soil and Water Contamination Risks

Chemical residues can persist in the environment, leading to long-term contamination:

- **Soil Contamination**:
  - Repeated applications can lead to accumulation of pesticide residues.
  - Affects soil health, microbial activity, and plant root development.
  - Some pesticides may bind tightly to soil particles, while others leach into groundwater.
- **Water Contamination**:
  - Pesticides may enter surface or groundwater through runoff, leaching, or accidental spills.
  - Contaminated water affects irrigation quality, aquatic ecosystems, and drinking water sources.

Mitigation:

- Avoid application before rainfall.
- Use proper containment and disposal methods.
- Adopt precision application techniques and soil-conserving practices.

Human Health Risks and Safety Measures

Exposure to chemical pesticides can pose risks at various stages—mixing, application, or handling residues:

- **Acute Health Effects**:
  - Skin and eye irritation, dizziness, nausea, and respiratory problems due to direct contact or inhalation.
- **Chronic Health Effects**:
  - Long-term exposure may lead to cancer, hormonal disruption, neurological issues, or reproductive problems.

Safety Measures:

- Use of Personal Protective Equipment (PPE) such as gloves, masks, goggles, and protective clothing.
- Proper storage: Store chemicals in original containers, away from food and water.
- Safe mixing and disposal: Avoid spills, follow label instructions, and dispose of containers safely.
- Training and awareness: Educate farmers and applicators on first aid, poison control, and safety handling practices

5. Resistance Management

Mechanisms of Resistance Development

Resistance occurs when a population of plant pathogens becomes less sensitive or immune to a chemical control agent after repeated exposure. It is a result of natural selection, where resistant strains survive and reproduce.

Key mechanisms include:

1. **Target Site Modification**
   - Mutation in the pathogen's biochemical target (e.g., enzyme) reduces the binding of the chemical (e.g., resistance to QoI fungicides by altering the cytochrome b gene).
2. **Increased Efflux or Decreased Uptake**
   - Pathogens pump the chemical out of their cells or prevent its entry.

3. **Enhanced Metabolism**
    - Pathogens break down or detoxify the chemical before it can act.
4. **Bypass of Metabolic Pathways**
    - The pathogen alters its metabolic route to avoid the pathway inhibited by the chemical.
5. **Overexpression of Target Proteins**
    - The pathogen produces excessive amounts of the target protein, diluting the chemical's effect.

Strategies to Mitigate Resistance

To slow down or prevent resistance development, integrated and proactive strategies must be followed:

1. **Rotation of Chemicals with Different Modes of Action**
    - Use fungicides, bactericides, or nematicides from different chemical groups (FRAC codes) in sequential applications.
2. **Use of Chemical Mixtures**
    - Combine two or more chemicals with different mechanisms of action. At least one should have a multi-site mode (e.g., mancozeb + metalaxyl).
3. **Limit the Number of Applications per Season**
    - Reduce selection pressure by minimizing chemical exposure.
4. **Use Full Recommended Dose**
    - Sub-lethal doses can encourage survival of resistant strains.
5. **Combine with Non-Chemical Methods**
    - Integrate with biological control, resistant varieties, crop rotation, and cultural practices to reduce dependence on chemicals.
6. **Avoid Sole Reliance on Systemic Products**
    - Systemic products are more prone to resistance development than contact (multi-site) fungicides.

Monitoring and Management Practices

Regular monitoring and adaptive management are crucial to detect early signs of resistance and modify strategies accordingly.

1. **Field Scouting and Disease Surveillance**
    - Observe for poor disease control despite proper application (may indicate resistance).

2. **Pathogen Sensitivity Testing**
    - Conduct lab assays to test pathogen response to chemicals.
3. **Record-Keeping**
    - Maintain detailed records of:
        - Chemical use (active ingredient, rate, date)
        - Disease incidence and severity
        - Crop history and rotation
4. **Farmer and Extension Worker Training**
    - Educate on the importance of resistance management and practical strategies.
5. **Use Decision Support Tools and Forecasting Systems**
    - Time chemical applications based on disease risk models to reduce unnecessary spraying.

6. Regulatory and Legal Aspects

Overview of Relevant Regulations and Guidelines

Chemical pesticides are regulated to ensure they are safe and effective when used according to prescribed guidelines. Key regulatory aspects include:

1. **Registration and Approval**
    - Before a pesticide can be sold or used, it must be registered with national authorities (e.g., CIB&RC in India – Central Insecticides Board and Registration Committee).
    - Registration is based on:
        - Toxicity data
        - Environmental impact
        - Efficacy against target pests
2. **Pesticide Classification**
    - Based on toxicity level (e.g., red, yellow, blue, and green labels in India).
    - Classified into restricted, banned, and approved pesticides.
3. **International Standards**
    - Adherence to FAO, WHO, and Codex Alimentarius guidelines for maximum residue limits (MRLs).
    - Import/export laws also require compliance with global standards.
4. **Environmental Protection Acts**
    - Use of pesticides is governed under national laws like:
        - Insecticides Act, 1968 (India)

- Environmental Protection Act, 1986
- Pollution Control Board norms

Label Interpretation and Compliance

Understanding and following the **pesticide label** is both a legal requirement and a critical safety measure.

A pesticide label includes:

- Trade name and active ingredient
- Manufacturer's name and registration number
- Toxicity classification and hazard symbols
- Recommended crop and target pests
- Dosage and dilution instructions
- Precautions during use
- First-aid instructions in case of poisoning
- Storage and disposal instructions
- Pre-harvest interval (PHI) and re-entry interval (REI)

Using a pesticide against a crop or pest not listed on the label is a legal violation.

Reporting and Record-Keeping Requirements

Maintaining accurate records of pesticide usage is essential for regulatory compliance, safety audits, and resistance management.

Records should include:

- Name of pesticide, active ingredient, and batch number
- Date and time of application
- Treated crop and target disease
- Dosage, method, and equipment used
- Name of the applicator
- Weather conditions during application
- PPE used and safety measures followed

Reporting obligations may include:

- Notifying authorities in case of pesticide spills, poisoning, or adverse effects.
- Submitting usage data for government audits or subsidy schemes.
- Providing records during inspections by regulatory bodies.

8. Conclusion

The safe and effective use of chemical controls in plant disease management relies on a deep understanding of their principles, application practices, resistance management, and adherence to legal regulations. Chemical agents

such as fungicides, bactericides, and nematicides play a vital role in Integrated Disease Management (IDM) by providing targeted and timely protection against pathogens. However, their success depends on proper application methods, accurate timing and dosage, use of personal protective equipment (PPE), and regular maintenance of equipment to minimize risks to human health and the environment. Resistance development among pathogens is a growing concern, and it can be mitigated through strategies like rotating chemicals with different modes of action, using mixtures, and integrating non-chemical control methods. Additionally, compliance with regulatory frameworks, careful label interpretation, and diligent record-keeping are essential for ensuring legal use and environmental safety.

Looking ahead, the future of safe chemical control in agriculture lies in greater adoption of precision farming technologies, improved training for farmers and applicators, and the development of newer, low-toxicity, and target-specific chemical formulations. There is also a need for stronger integration of chemical control with biological and cultural practices, supported by real-time disease forecasting and decision support systems. Continued research, policy support, and public awareness are crucial to enhancing the sustainability and safety of chemical use in crop protection

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CHAPTER - 20

ECOLOGICAL IMPACT OF INTEGRATED DISEASE MANAGEMENT (IDM) PRACTICES

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Abstract

Integrated Disease Management (IDM) practices aim to control plant diseases using a combination of cultural, biological, and chemical methods to minimize their impact on crops while maintaining ecological balance. This chapter explores the ecological implications of IDM practices by examining their effects on biodiversity, soil health, and ecosystem stability. It discusses how IDM practices can enhance resilience against disease outbreaks, reduce reliance on chemical inputs, and promote sustainable agricultural systems. The chapter also highlights case studies demonstrating the successes and challenges of implementing IDM strategies in various agricultural contexts.

1. Introduction

Integrated Disease Management (IDM) is a holistic approach to plant disease control that combines multiple strategies—cultural, biological, mechanical, genetic, and chemical—in a coordinated manner to manage diseases effectively, economically, and sustainably. The primary goal of IDM is to minimize the economic losses caused by plant diseases while reducing the reliance on chemical pesticides, thereby promoting environmental health, food safety, and long-term agricultural productivity.

Traditionally, plant disease management relied heavily on single-method approaches, especially the use of chemical fungicides and bactericides. Although often effective in the short term, these methods can lead to unintended consequences such as the development of resistant pathogen strains, residue accumulation in crops, environmental pollution, and harm to beneficial organisms. In addition to chemical control, traditional methods have also included cultural practices (like crop rotation and sanitation), the use of resistant varieties, and mechanical removal of infected plants.

The rationale for integrating multiple approaches in IDM arises from the complexity of disease dynamics in agricultural ecosystems. No single

method can offer complete, long-term protection. By combining diverse strategies in a complementary way, IDM enhances disease suppression, delays resistance development, and reduces the need for intensive chemical input. This integrated framework also aligns with modern sustainability goals, regulatory standards, and the increasing demand for safe and residue-free agricultural produce.

2. Components of IDM

Cultural Practices

Cultural practices form the first line of defense in IDM by modifying the environment to suppress disease development and reduce pathogen spread.

- **Crop Rotation**: Disrupts the life cycles of soil-borne pathogens by alternating crops that are not susceptible to the same diseases.
- **Resistant Varieties**: Use of disease-resistant or tolerant crop cultivars reduces dependency on chemical treatments.
- **Planting Time Adjustment**: Avoiding peak periods of disease pressure by changing sowing dates.
- **Proper Spacing and Pruning**: Enhances air circulation and reduces humidity, lowering the risk of foliar diseases.
- **Sanitation**: Removal of infected crop residues, weeds, and volunteer plants to eliminate sources of inoculum.
- **Soil Management**: Practices like deep ploughing, solarization, and organic amendments can suppress soil-borne pathogens.

Biological Control

Biological control uses **living organisms to suppress disease-causing pathogens** in a natural and environmentally friendly way.

- **Beneficial Microorganisms**: Use of antagonistic fungi and bacteria like *Trichoderma spp.*, *Pseudomonas fluorescens*, and *Bacillus subtilis* that compete with or inhibit pathogens.
- **Biocontrol Agents (BCAs)**: Commercial formulations of beneficial organisms applied as seed treatments, soil applications, or foliar sprays.
- **Predators and Parasitoids**: Though more commonly used against insect pests, some natural enemies also help suppress vectors of plant diseases.
- **Mycorrhizal Fungi**: Improve nutrient uptake and enhance plant resistance to pathogens.

Biological control is eco-friendly and can be used in conjunction with chemical and cultural methods to enhance overall effectiveness.

Chemical Control

Chemical control remains a necessary component of IDM, especially under high disease pressure, but it is applied more judiciously:

- **Reduced Use and Targeted Application**: Use only when needed, based on monitoring and threshold levels.
- **Selective and Safe Chemicals**: Prefer low-toxicity, systemic, or contact fungicides and bactericides with minimal impact on non-target organisms.
- **Avoidance of Overuse**: Reduces the risk of resistance development and environmental contamination.
- **Proper Timing and Dose**: Ensures maximum efficacy and minimal residues.

Chemical control should always be integrated with other IDM components and never used as a sole method.

Monitoring and Decision-Making

Accurate monitoring and timely decision-making are central to the success of IDM.

- Scouting and Surveillance: Regular field inspections to detect early disease symptoms.
- Use of Threshold Levels: Decisions to apply chemicals or other controls are based on the level of disease incidence or severity.
- Forecasting Systems and Disease Models: Weather-based models help predict disease outbreaks, allowing for timely interventions.
- Record-Keeping: Tracking of disease trends, pesticide use, and outcomes helps refine future management strategies.

3. Ecological Impact

Impact on Non-Target Organisms

IDM minimizes the adverse effects on non-target organisms by reducing unnecessary chemical applications. Broad-spectrum pesticides often harm beneficial insects, birds, aquatic species, and other wildlife. In contrast, IDM's selective approach:

- Preserves natural enemies of pests (e.g., lady beetles, spiders, parasitoids).
- Maintains ecological interactions crucial for pest suppression and pollination.

- Reduces pesticide runoff that affects aquatic biodiversity.

Role in Promoting Beneficial Microorganisms

Biological control agents such as *Trichoderma spp.*, *Bacillus subtilis*, and *Pseudomonas fluorescens* used in IDM enhance microbial diversity and activity in the rhizosphere. These beneficial microbes:

- Compete with or antagonize pathogens.
- Induce systemic resistance in plants.
- Improve nutrient solubilization and uptake.

Case Studies: Effects on Insect Populations and Soil Flora

- **IPM in Apple Orchards**: Reduced chemical use led to recovery of predator mite populations and balanced pest-predator dynamics.
- **Rice-Wheat Systems**: Use of biocontrols and pheromone traps protected pollinators and predatory insects while maintaining yields.
- **Organic Vegetable Farming:** Integration of compost and microbial inoculants enhanced fungal and bacterial diversity in soils.

3.2 Soil Health

Effects on Soil Structure and Fertility

Repeated chemical pesticide use can degrade soil structure and reduce fertility. IDM, through the inclusion of organic amendments, reduced tillage, and crop rotation:

- Enhances soil aggregation and aeration.
- Promotes organic matter buildup.
- Prevents compaction and erosion.

Influence on Microbial Communities

Soil microbial communities are essential for nutrient cycling and plant health. IDM practices, especially the use of microbial biopesticides and organic inputs:

- Support a diverse and functional microbial population.
- Suppress soil-borne pathogens naturally.
- Improve root-zone health and plant-microbe symbioses.

Long-term Sustainability and Soil Resilience

IDM builds soil resilience by maintaining microbial and nutrient balance. Sustainable soil health outcomes include:

- Enhanced plant vigor and reduced disease susceptibility.
- Increased water-holding capacity and nutrient-use efficiency.
- Reduced reliance on synthetic fertilizers and fumigants.

3.3 Ecosystem Stability

Contributions to Ecosystem Services

By conserving biodiversity and promoting ecological balance, IDM contributes to:

- **Pollination**: Protects insect pollinators by avoiding harmful pesticides.
- **Nutrient Cycling**: Encourages microbial-driven nutrient transformations.
- **Natural Pest Regulation**: Maintains predator-prey balance.

Resistance to Disease Outbreaks

Diversified cropping systems and biologically active soils promoted under IDM reduce pathogen survival and multiplication, increasing the ecosystem's natural resistance to epidemics. Early detection and threshold-based interventions also help avert major outbreaks.

Examples of Successful IDM Implementation in Diverse Ecosystems

- **Tomato Production**: Use of resistant cultivars, solarization, and biocontrols significantly reduced wilt and root-knot nematode problems.
- **Banana Plantations:** IDM practices including mulching, cover cropping, and biocontrols reduced Fusarium wilt and improved biodiversity.
- **Maize Systems**: Integration of cultural, chemical, and biological measures successfully managed downy mildew while conserving predator insects.

4. Benefits and Challenges

Advantages of IDM for Ecological Balance

IDM promotes ecological harmony by integrating multiple, low-impact strategies for disease management, leading to several benefits:

- **Conservation of Biodiversity**: By reducing reliance on broad-spectrum chemical pesticides, IDM helps preserve beneficial insects, pollinators, soil organisms, and natural predators.
- **Environmental Protection**: Minimal chemical runoff and residue accumulation safeguard water quality, reduce air pollution, and protect non-target habitats.
- **Enhanced Soil Health**: Practices like organic amendments, crop rotation, and use of biocontrols improve soil microbial diversity, structure, and fertility.

- **Reduced Resistance Development**: Rotating control methods (e.g., chemical, biological, cultural) delays the development of pathogen resistance, making disease control more durable.
- **Sustainable Productivity**: IDM contributes to long-term agricultural resilience by improving crop health and yield stability under variable environmental conditions.

Potential Risks and Unintended Consequences

Despite its strengths, IDM can sometimes lead to unintended consequences if not carefully planned and executed:

- **Improper Biocontrol Use**: Introduction of non-native or poorly tested biocontrol agents may disrupt local ecosystems or outcompete native species.
- **Inconsistent Results**: Biological and cultural methods may be less reliable under certain environmental conditions or high disease pressure, leading to variable efficacy.
- **Residual Chemical Dependency**: In high-value crops or severe outbreaks, farmers may still resort to chemical controls, potentially undermining IDM principles.
- **Knowledge Gaps**: Inadequate understanding of pathogen dynamics, cropping systems, and microbial interactions can limit the effectiveness of IDM strategies.

Economic and Practical Considerations

The implementation of IDM involves several economic and practical factors that influence its adoption:

- **Cost-Benefit Balance**: While IDM can reduce long-term input costs, initial investments in diagnostics, resistant varieties, or biocontrol agents may be high.
- **Training and Awareness**: Effective implementation requires farmer education, training in pest monitoring, and knowledge of integrated practices.
- **Infrastructure Needs**: Availability of disease forecasting tools, quality biopesticides, and resistant seeds can limit access in rural or resource-poor areas.
- **Time and Labor**: IDM practices such as regular scouting, record-keeping, and cultural interventions may demand more time and labor than conventional methods.

5. Conclusion

Integrated Disease Management (IDM) stands out as a comprehensive and ecologically responsible approach to managing plant diseases. Its implementation significantly reduces the environmental footprint of agriculture by promoting biodiversity, enhancing soil health, and strengthening ecosystem services such as pollination, nutrient cycling, and natural pest regulation. Through the strategic use of cultural, biological, chemical, and decision-support tools, IDM minimizes the adverse impacts on non-target organisms and helps maintain ecological balance across diverse cropping systems.

By supporting soil microbial diversity and reducing chemical inputs, IDM contributes to improved soil structure, fertility, and long-term sustainability. Case studies across different regions have demonstrated its effectiveness in stabilizing ecosystems and reducing the risk of disease outbreaks without compromising yield or quality.

As agriculture faces increasing pressures from climate change, pesticide resistance, and resource degradation, IDM offers a viable path forward. It aligns with the goals of sustainable farming by integrating science-based practices that are economically sound, environmentally safe, and socially acceptable. Moving ahead, the broader adoption of IDM will require continued investment in research, farmer education, policy support, and development of locally adapted technologies. Ultimately, IDM is not just a disease management strategy, but a cornerstone of resilient and sustainable agriculture.

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CHAPTER - 21
POLICY AND REGULATORY FRAMEWORKS IN DISEASE MANAGEMENT

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Abstract:
The effective management of diseases requires a robust framework of policies and regulations that guide public health responses, ensure equitable access to healthcare, and promote the development and implementation of medical interventions. This chapter explores the critical role of policy and regulatory frameworks in disease management, focusing on the historical evolution, current practices, and future directions. It examines key elements such as health policies, regulatory agencies, international guidelines, and the impact of these frameworks on disease prevention, control, and treatment. The chapter also highlights case studies of successful policy implementations and identifies challenges faced by policymakers in adapting to emerging health threats. By providing a comprehensive overview, this chapter aims to offer insights into how effective policy and regulatory mechanisms can enhance disease management and contribute to global health security.

1. Introduction

Plant diseases pose a significant threat to global food security, agricultural productivity, and rural livelihoods. Effective disease management is therefore a cornerstone of sustainable crop production. Traditionally, plant diseases have been managed through chemical pesticides, resistant crop varieties, cultural practices, and biological control methods. However, the increasing complexity of agricultural systems, the emergence of new pathogens, climate change, and concerns over environmental and human health have necessitated more integrated and regulated approaches to disease control.

In this context, policy and regulatory frameworks play a vital role in guiding the safe, effective, and sustainable use of disease management tools. These frameworks ensure that plant protection products are evaluated for efficacy

and safety, facilitate the responsible registration and use of agrochemicals and biological agents, and support research, education, and monitoring programs. Regulations also protect biodiversity, promote sustainable farming practices, and help harmonize international trade by ensuring compliance with phytosanitary standards.

A well-structured regulatory environment not only minimizes risks associated with pesticide misuse and environmental contamination but also fosters innovation in developing safer and more effective disease control technologies. As such, integrating policy support with scientific strategies is essential for advancing modern plant disease management in a sustainable and legally compliant manner.

2. Historical Evolution of Disease Management Policies

Early Frameworks and Their Impact

In the early 20th century, disease management largely depended on cultural practices and rudimentary chemical treatments such as sulfur and copper-based compounds. The introduction of synthetic pesticides in the 1940s, particularly after World War II, marked a major shift in plant protection policies. Early frameworks focused primarily on:

- **Product efficacy and pest control**: Ensuring that pesticides were effective in managing specific diseases.
- **Basic safety protocols**: Initial attempts to mitigate acute toxicity risks to humans and livestock.
- **National agricultural laws**: Countries began enacting their first plant protection acts (e.g., the Insecticides Act of 1968 in India) to regulate pesticide manufacturing and use.

These early policies significantly increased agricultural productivity but often overlooked long-term environmental and health implications.

Major Milestones and Reforms

As awareness grew around the negative consequences of indiscriminate pesticide use—such as resistance development, environmental pollution, and health hazards—major reforms began shaping modern disease management policy. Key milestones include:

1. **1962 – Publication of *Silent Spring* by Rachel Carson**
   This landmark book exposed the dangers of pesticide overuse and sparked global environmental consciousness, prompting governments to reevaluate regulatory standards.

2. **1970s–1980s – Establishment of Regulatory Bodies**
   Agencies such as the U.S. Environmental Protection Agency (EPA) and the European Food Safety Authority (EFSA) were formed to oversee the registration, regulation, and monitoring of pesticides.
3. **1990s – Emphasis on Integrated Pest Management (IPM)**
   Governments and institutions began promoting IPM and IDM strategies, integrating chemical, biological, and cultural methods for sustainable disease control.
4. **Rotterdam and Stockholm Conventions (1998–2001)**
   These international treaties addressed the trade and use of hazardous chemicals, ensuring informed consent and responsible handling of persistent organic pollutants (POPs), including certain fungicides.
5. **Codex Alimentarius and WTO-SPS Agreement**
   Global food safety standards and the Sanitary and Phytosanitary Measures Agreement ensured harmonized plant protection regulations and fair trade practices based on scientific risk assessment.
6. **Recent Advances**
   - **Biopesticide regulation**: Many countries have developed fast-track procedures for registering low-risk biological agents.
   - **Data-driven policies**: Use of disease forecasting models and environmental monitoring tools in regulatory decision-making.
   - **Bans and restrictions**: Phasing out of highly hazardous pesticides (e.g., DDT, methyl bromide) in favor of eco-friendly alternatives

3. Key Components of Policy and Regulatory Frameworks

Health Policies and Their Objectives

Plant disease management policies are closely aligned with broader **public health and environmental safety goals**. The core objectives include:

- **Protecting human health**: Ensuring that chemical control agents (like fungicides and bactericides) do not pose acute or chronic health risks to farmers, consumers, and nearby populations.
- **Food safety**: Regulating pesticide residue levels through Maximum Residue Limits (MRLs) to ensure food remains safe for consumption.

- **Environmental protection**: Minimizing contamination of soil, water, and air, and safeguarding non-target organisms including pollinators and beneficial insects.
- **Sustainable agriculture**: Encouraging reduced pesticide use, promoting integrated approaches, and supporting the development of low-risk biopesticides and natural products.

These policies aim to strike a balance between agricultural productivity and ecological responsibility.

Regulatory Agencies and Their Roles

National and regional regulatory agencies are tasked with the formulation, implementation, and enforcement of plant protection policies. Their roles include:

- **Registration and evaluation of plant protection products**: Agencies conduct risk assessments to determine the efficacy, toxicity, environmental impact, and permissible use conditions.
- **Labeling and usage guidelines**: Ensuring proper instructions, safety warnings, and dosage information are clearly provided for users.
- **Monitoring and surveillance**: Overseeing post-market environmental and residue monitoring programs to detect misuse or resistance.
- **Farmer education and training**: Providing guidelines and support to promote safe application and compliance.

Examples of regulatory agencies:

- **India**: Central Insecticides Board and Registration Committee (CIBRC)
- **USA**: Environmental Protection Agency (EPA)
- **EU**: European Food Safety Authority (EFSA)
- **FAO/WHO**: Joint Meeting on Pesticide Residues (JMPR)

International Guidelines and Collaborations

In a globally interconnected food system, **international cooperation** ensures harmonized standards for trade, safety, and environmental stewardship. Key international frameworks include:

- **Codex Alimentarius (FAO/WHO)**: Sets international food safety standards including MRLs and pesticide use guidelines.

- **WTO-SPS Agreement**: Ensures that sanitary and phytosanitary measures (e.g., pest and disease control regulations) are science-based and non-discriminatory in trade.
- **Rotterdam Convention**: Facilitates informed consent procedures for international trade in hazardous chemicals.
- **Stockholm Convention**: Restricts or bans persistent organic pollutants (POPs) that pose risks to human and environmental health.
- **OECD Guidelines**: Provide testing standards for pesticide registration, used by many countries to harmonize data requirements.

5. Challenges and Future Directions

Adapting to Emerging Health Threats

One of the most pressing challenges is the rise of new and re-emerging plant pathogens, many of which are influenced by changing climatic conditions, increased global trade, and shifts in cropping patterns. These evolving threats demand:

- **Faster regulatory responses**: Streamlining the approval of new disease control agents (e.g., biopesticides, nanotechnologies) without compromising safety.
- **Enhanced surveillance systems**: Strengthening disease monitoring, diagnostics, and forecasting tools to detect outbreaks early.
- **Cross-sector collaboration**: Integrating plant health with One Health approaches to manage interconnected threats to plants, animals, and humans.

Regulatory frameworks must be flexible and science-responsive, capable of quickly adapting to novel risks while maintaining public trust.

Addressing Disparities and Ensuring Equity

Regulatory systems often face challenges in reaching smallholder farmers and marginalized communities, who may lack access to information, safe products, or government support. Key issues include:

- Limited access to registered biopesticides or improved inputs in rural areas.
- Knowledge gaps in understanding pesticide labels, safety protocols, and integrated management practices.
- Gender and regional disparities in extension services and policy influence.

Future frameworks must emphasize inclusive policy development, ensuring that disease control solutions are equitable, accessible, and culturally appropriate. This may involve community-led initiatives, capacity building, and subsidies for safe alternatives to hazardous chemicals.

Innovations in Policy and Regulation

To overcome current limitations and build a more robust regulatory future, several innovations are emerging:

- **Digital platforms and mobile apps**: For real-time disease diagnosis, pesticide use guidance, and reporting adverse effects.
- **Risk-based and adaptive regulation**: Policies that shift from rigid, hazard-based models to more holistic, risk assessment approaches.
- **Harmonization of international standards**: Simplifying trade and registration processes across borders through shared protocols and mutual recognition agreements.
- **Incentivizing green technologies**: Offering fast-track registration or subsidies for low-risk, eco-friendly products and practices.
- **Public-private partnerships**: Leveraging industry innovation with public oversight to co-develop safe and effective solutions.

6. Conclusion

The development and implementation of robust policy and regulatory frameworks are essential for ensuring the safe, effective, and sustainable management of plant diseases. Over the years, these frameworks have evolved from simple pesticide use guidelines to comprehensive systems that integrate health, environmental, and trade considerations. They play a crucial role in protecting human health, preserving biodiversity, and supporting agricultural productivity.

Key components of these frameworks include health-focused objectives, the involvement of dedicated national and international regulatory bodies, and the adoption of globally harmonized standards. Despite their successes, several challenges remain, including the need to respond quickly to emerging plant health threats, ensure equitable access to safe disease control tools, and embrace innovations in risk assessment and regulatory processes.

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CHAPTER 22

CASE STUDIES- SUCCESSFUL IDM PROGRAMS WORLDWIDE

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ABSTRACT

Plant diseases, caused by various pathogens such as fungi, bacteria, viruses and nematodes, significantly impact global agriculture, leading to reduced crop yields and compromised food security. These diseases not only threaten the economic stability of farming communities but also have far-reaching consequences for human lives. The loss of crops due to plant diseases can lead to increased food prices, reduced availability of certain foods, and even contribute to malnutrition in vulnerable populations. In extreme cases, widespread crop failures have historically led to famines, with devastating effects on human health and society. Integrated Plant Disease Management (IPDM) Programs have emerged as a comprehensive approach to combat plant diseases. These programs combine multiple strategies to manage plant diseases in an environmentally sustainable and economically viable manner. The implementation of IPDM programs can significantly reduce the incidence and the severity of plant diseases, thereby safeguarding food production, protecting livelihoods and enhancing the resilience of agricultural systems against future challenges.

Keywords: Integrated Disease Management, Genetic resistance, Plant Diseases, Phytopathogens, Cultivars

INTRODUCTION

Plant diseases are important biotic constraints resulting in significant crop losses worldwide. IDM programs are strategies that are used to counter plant diseases on an international scale. They are formulated by combining multiple approaches such as cultural, genetic, chemical and biological methods in a coordinated and sustainable manner. Major components of disease management are Host plant resistance, cultural practices, biological

control and chemical control [1-5]. Let us delve into the examples of successful IDM programs against specific plant diseases implemented by different countries on a global scale.

1. Wheat IDM Program -

Causative Agent- *Pyricularia* species.

Location - Great Plains & Pacific Northwest.

This disease was first reported in Brazil in 1985 [6] and in the neighbouring states of Sao Paulo and Mato Grosso do Sul in 1986 [7]. It started spreading to other neighbouring countries. In Feb 2016, it was reported in Bangladesh [8] for the first time thereby bringing the attention of the entire Asian community of plant pathologists onto this disease. According to reports, a total of 7 million hectares in India, Pakistan, and Bangladesh are susceptible to wheat blast [9].

The disease is typically caused by the general spread of air-borne spores and infected seeds [10,11]. Scattered patches start to appear during the reproductive phase of the crop. These patches then coalesce and spikes are formed in silvery color [12]. The extent of damage depends on the intensity and timing of the infection [13].

IDM Program is targeted on combining Resistant varieties, Cultural practices like timely sowing and crop rotation and targeted fungicide use altogether. Infected seeds are prone to be viable for about 22 months [14].

This viability can be destroyed by fungicidal seed treatment [15]. At present, five commercially registered fungicides are being used to treat wheat blast. Their active ingredients being Iprodione, Fluazinam with Thiophanate-methyl, Difenoconazole, and Difenoconazole mixed with Metalaxyl-M and Thiamethoxam, and Carboxin with Thiram [16].

Genetic resistance of wheat cultivars is also an essential parameter to obtain disease management ability. Out of he 100 blast resistance genes mapped in rice by researchers, only 9 are been found to be blast resistant- *Rmg1, Rmg2, Rmg3, Rmg4, Rmg5, Rmg6, Rmg7, Rmg8,* and *R m g G R119* [13].

Cultural practices like deep ploughing could bring down the initial pathogen inoculum present in crop stubble [17]. Reduction of crop stubble and crop rotation are also followed to reduce the onset of disease.

This protocol emphasized the early detection of the disease and prompt action was taken to prevent the spread of it. Proper field management including proper spacing between plants and good air circulation ensured

reduced growth of pathogens. Farmers were also trained to adopt these IDM practices efficiently. Success of this program resulted in the reduced reliance on chemical treatments and improved sustainability in wheat production.

Rice IDM Program-

Causative Agent - *Magnaporthe oryzae (*fungus).

Location- Philippines, Vietnam and Indonesia.

Rice is an important staple food crop all over the world. One of the most devastating diseases of rice includes Rice blast that accounts for over 30% of production losses globally which is roughly equivalent to feeding 60 million people [18] . Therefore keeping these figures in mind, it is essential to tackle this disease with iron hands.

The control of rice blast disease is generally done by usage of genetically resistant strains. But increased resistance by blast strains has rendered this method insufficient [19] . Many resistant genes such as *Piz*, *Piz-t*, *Pit*, *Pik*, *Pik-m*, *Pik-p*, *Pita*, *Pita-2*, and *Pib* were isolated and used as resistant donors in various breeding programs to defer the phytopathogenic effects of the disease pathogen [25].

Usage of fungicides such as Carpropamid ((1R*, 3S*)-2, 2-dichloro-N-[1-(4-chlorophenyl) ethyl]-1-ethyl-3-methylcyclopropane-carboxamide}) was found to be effective exhibiting systemic properties [20]. Use of biocontrol agents such as *Trichoderma spp* have also been employed. Usage of bioactive compounds such as Blasticidin- C [22] and Kasugamycin [23] obtained from Streptomyces spp have also been reported to treat the disease [24].

Cultural methods such as segregation of infected grains, good field hygiene and avoiding broadcast sowing are followed to reduce the onset of the disease [21]. Timely planting to avoid peak disease periods and proper water management to reduce humidity around plants are taken care of.

Farmers are taught about these integrated approaches and explaining the effects of judicious usage of fungicides. Successful implementation of such practices were ensured by field demonstrations and participatory methods [21]. The success of this program resulted in the significant reduction in the use of chemical fungicides and increase in the yield of rice crops.

Potato IDM Program -

Causative Agent - *Phytophthora infestans*

Location - UK and Netherlands.

Historically speaking, this pathogen was responsible for the outbreak of one of the most destructive plant diseases of all times, causing the Great Irish Famine of the 1940s - 1950s. Even now, this disease poses a great risk for potato breeders and farmers leading to decreased production. The disease appears as irregular pale green lesions near the tips and margins of leaves which then rapidly grows into large brown to black necrotic spots [28].

Uncovered infected dumps were responsible to be primary inoculum sources of the pathogen. Hence, farmers were thoroughly informed about a nationwide regulation to cover dumps organized by the Masterplan Phytophthora, launched by the Agricultural and Horticultural Organization LTO-Netherland in 1999 [26].

In the UK, list of approved fungicides include Benthiavalicarb-isopropyl and Fluopicolide, Mandipropamid, Amisulbrom and Ametoctradin for respective breeding seasons [27].

In Denmark, application of inoculum in the field is done to test the resistance to blight after partial defoliation, and methods were closely related to the EUCABLIGHT protocol [*www.eucablight.org*] [27]. This field test for resistance against tuber blight also includes the cultivar-specific effect of avoidance of inoculum along with intrinsic tuber resistance. A moving graph concept was developed in India by Singh et al. [29] using a computerized forecasting model 'JHULSACAST' for western UP.

Various cultivational approaches such as integration of Biocontrol agents like *Bacillus subtilis,* Crop Rotation and prompt removal of infected plant debris are advised. Timely field sanitation and regular weather monitoring were carried out. Timely action through real-time disease advisories to farmers helps in keeping the disease at check. Reduced dependency of fungicides and increased potato yield were the benefits enjoyed as a result of this program.

Grapevine IDM Program -

Causative Agent- *Plasmopara viticola* (the causal agent of downy mildew) and *Erysiphe necator* (the causal agent of powdery mildew)

Location - USA and European Vineyards.

Grapevine is an important cash crop used for the production of wines and table grapes. This disease got introduced from America in the 19th century. Powdery Mildew disease appears as white powdery outgrowth on both sides

of the leaf while Downy Mildew affects the lower part of the leaves. Powdery mildew results in reducing the aesthetic value of the plants while Downy Mildew results in plant death.

Planting of hybrids even while providing fair resistance to the infection resulted in poor wine quality. Therefore, the best strategy to control mildews is to prevent or stop the spreading of spores and their germination [30,31] and hence it was followed strictly. Improving Canopy management, soil health improvement and sanitary practices were followed [32,33].

Impactful disease models were used. They were complex advisory systems such as Vitimeteo *Plasmopara* (which is used in Switzerland and southern Germany) [34] or are used to develop grower recommendations that are transmitted through technical bulletins (i.e., Epi and Milvit in France [35]). Use of predatory insects and fungi to manage disease outbreak and introduction of resistant varieties were key measures to manage the disease. Its success resulted in the production of enhanced quality of wine grapes and sustainable vineyard management.

Tomato IDM Program-

Causative Agent - Tomato Spotted Wilt Virus.

Location - Florida.

This disease is one of the most important diseases that not only affects tomatoes but also peanuts, peeper and many ornamental plants as well [36]. This disease is originated in Australia and spread to different parts of the world [37]. The virus is said to replicate in an insect named F. occidentalis, thereby acting as a viral host [38]. The disease the uppermost leaves appear bronzed with small brown lesions and infected plants form small brown necrotic spots and/or chlorotic ring spots on the foliage.

Multiple management practices are considered superior for disease management against individual ones as they aid in cumulative suppression of the disease [39]. Resistant Cultivar varieties like Southern Runner [40] and Georgia Green [41] exhibited moderate resistance towards the disease while the use of seed treatment combinations of Carboxin, Captan, and Pentachloronitrobenzene (PCNB) increased plant stands thereby reducing the onset of the disease [42].

Row Planting Pattern, reduced Tillage and Weed Control are other cultivation techniques utilized to mitigate the disease [39]. Usage of healthy seedlings, roughing of infected plants upto 45 days of planting and growing

Sorghum or Maize as a barrier crop are employed as well to control the disease.

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