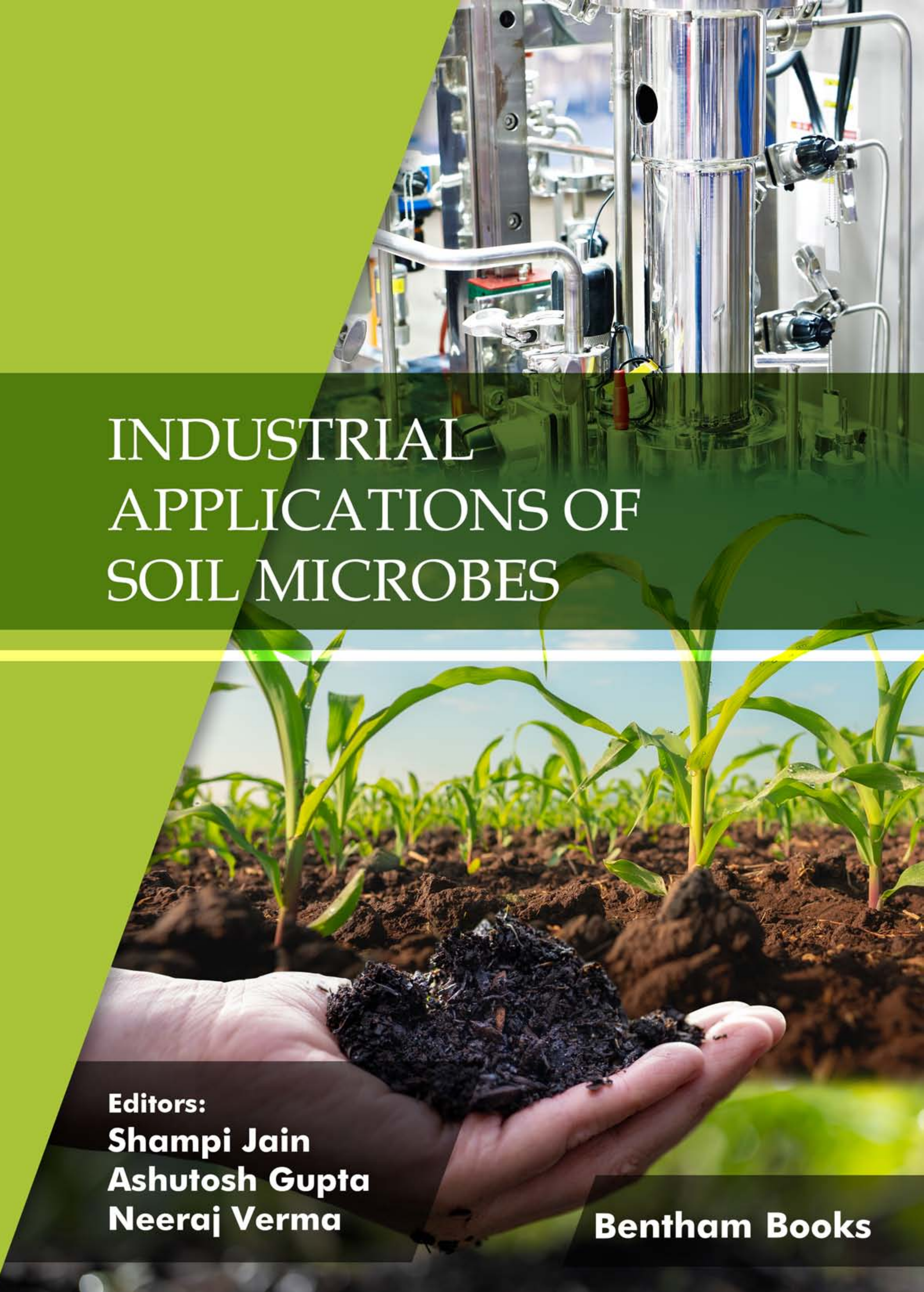
INDUSTRIAL APPLICATIONS OF SOIL MICROBES



Editors:
Shampi Jain
Ashutosh Gupta
Neeraj Verma

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Industrial Applications of Soil Microbes

(Volume 4)

Edited by

Shampi Jain

Ashutosh Gupta

&

Neeraj Verma

Department of Agriculture Science, AKS University
Satna, Madhya Pradesh
–485001 India

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FOREWORD

I, being an academician and researcher, feel very happy to write a foreword for the present book "Industrial Applications of Soil Microbes" volume 4. In the present scenario of environmental conditions, plant health and humans need for a sustainable life, therefore, it has become a need to find out solutions to increase healthy crop yield, medicines, food, *etc*.

In the past, we have used enormous chemical fertilizers to increase the crop yield to feed the increasing population of the world. But it has caused a loss to the physical structure of the soil and its chemistry. These chemicals have also caused chemical pollution to the soil and the environment. Therefore, there is a rise in various plant, animal and human diseases. Therefore, there is a need to revive the soil and the microorganisms living in a harmonious environment.

I am sure that the chapters written by various eminent experts in their field on mycorrhizae, fungi, bacteria and soul-borne viruses will not only provide basic information about these microorganisms but also present the recent development in this field. The metagenomic approach is also an important way to find out soil microbes in a very less time and is helpful in the study of these microbes. An interesting part is that metal-nanoparticle interaction with plants and soil microbes could also be a new source to improve crop yield.

I am sure that this book will be helpful to all the academicians, researchers, graduate and postgraduate students of agriculture as well as biotechnology and industrialists. I wish good luck to all the authors of the book and the editors.

B.A. Chopade
Dr. Babasaheb Ambedkar Marathwada University
&
Rashtriya Pramukh
Anusandhan Prakoshth
Bhartiya Shikshan Mandal
Nagpur (Mah), India

PREFACE

Soil microbes, including algae, fungi, bacteria, and nematodes, can cause significant plant diseases, resulting in substantial yield losses. Conversely, healthy soil contains a diverse array of microbes in a delicate balance. These microscopic and submicroscopic organisms are crucial not only for plant health but also for the well-being of animal life and the overall environment. Over the past 30 to 35 years, the field of soil microbiology has blossomed, particularly after key discoveries regarding nutrient cycles involving these microbes. The presence or absence of such microorganisms can influence nutrient cycling, soil conditions, and environmental health. A single gram of soil can host a multitude of microbes that interact with one another and their surroundings, driving various biogeochemical cycles. Mycorrhiza is a prime example of this interaction, where fungi partner with plant roots to enhance mineral nutrient uptake. Plants with mycorrhizal associations exhibit improved growth, development, and yield under favorable environmental conditions.

The discovery of secondary metabolites produced by microbes during their interactions with plants and the environment has revealed their significant benefits to human life. These metabolites are now employed across various industries, including pharmaceuticals, food and beverages, and textiles. Advances in biotechnology and genome sequencing have deepened our understanding of these microbes and their interactions with both plants and the environment. This growing knowledge continues to enhance our ability to harness the potential of these secondary metabolites for various applications.

This book's chapters delve into the dynamics of soil microbes, including bacteria, fungi, and mycorrhizae, while also highlighting the latest advancements in the field. The volume is divided into two parts: the first focuses on fundamental and advanced knowledge of mycorrhizae and their interactions with plants, while the second addresses other soil microbes, such as fungi, bacteria, and soil-borne viruses. Additionally, it explores the effects of metal nanoparticles on fungal and bacterial populations at the molecular level, aiming to enhance plant health, growth, and yield. Microbial genetic diversity plays a crucial role in the soil environment, and metagenomic analyses can uncover novel molecules for therapeutic, biotechnological, and sustainable agricultural applications.

We encourage students in biology, ecology, biogeochemistry, and soil science, as well as participants in online courses, engineers, foresters, biogeochemists, agronomists, biotechnologists, bioscience educators, and researchers worldwide to engage critically with our volumes for insights relevant to their respective fields.

With optimism, the editors of this volume welcome suggestions and comments to enhance the content for future editions.

Shampi Jain

Ashutosh Gupta

&

Neeraj Verma
Department of Agriculture Science, AKS University
Satna, Madhya Pradesh
–485001 India

List of Contributors

Amar P. Garg	Swami Vivekanand Subharti University, NH-58, Meerut, UP- 250005, India
Alok Kumar Varshney	Birla Institute of Scientific Research, Statue Circle, Jaipur, Rajsthan, India
	Department of BioEngineering, Birla Institute of Technology, Mesra, Ranchi, Jaipur Campus, Jaipur, Rajasthan, India
Ashwini A. Waoo	Department of Biotechnology, AKS University, Satna, 485001, MP, India
Abdul Raheem	Department of Botany, University of Lucknow, Lucknow-226 007, India
Asha Nabi	Plant Virology and Molecular Pathology Lab., Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, 190025, Jammu and Kashmir, India
B.A. Padder	Plant Virology and Molecular Pathology Lab, Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, 190025, Jammu and Kashmir, India
C.G. Sangeetha	Department of Plant Pathology, College of Horticulture, UHS Campus, G.K.V.K. Post, Bengaluru, 560065, India
Diwakar Bahukhandi	Retd. Principal Scientist, Mycology (Mushroom), Division of Mycology and Plant Pathology, ICAR-IARI, Pusa, New Delhi, India
Ekta Narwal	Department of Agriculture and Agri-informatics, Shobhit Institute of Engineering and Technology, Meerut, UP- 250110, India
F. Ahmad	508 BWT, Eros Garden, Charmwood Village, Faridabad, Haryana, India
G. Rajesha	ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad, 500030, Telangana, India
G.N. Chattopadhyay	Emeritus Professor, Seacom Skills University, Kendradangal, 731236, Birbhum, WB, India
Gourab Roy	M.B.B. College , Agartala-799004, Tripura, India
G.H. Dar	Centre for Advancement of Applied Sciences, Shalimar, 190025, Jammu and Kashmir, India
Humaira Rani	Lotus Institute of Pharmacy, Bareilly, 243123, India
Jairam Choudhary	Division of Organic Agriculture Systems, ICAR-Indian Institute of Farming System Research, Meerut, 250110, India
Lalit Mohan	Department of Microbiology, Ram Lal Anand College, University of Delhi, New Delhi, India
Mukul Sharma	Sri Aurobindo Institute of Pharmacy, Indore (M.P.), India
Mohammad Israil Ansari	Department of Botany, University of Lucknow, 226007, India
M. Hajong	Soil & Water Conservation Division, Simsanggre, Williamnagar, East Garo Hills, Meghalaya, 794111, India
Mohammad Danish	Department of Botany, A.M.U., Aligarh-202001 (Uttar Pradesh), India
Mohammad Amir	Department of Botany, University of Lucknow, Lucknow-226 007, India

Mohd Ashaq	Department of Botany, Govt Degree College, Thannamandi, District Rajouri, 185132, Jammu and Kashmir, India
Mushatq Ahmad	Centre for Advancement of Applied Sciences, Shalimar, 190025, Jammu and Kashmir, India
Mehraj D. Shah	Plant Virology and Molecular Pathology Lab, Division of Plant Pathology, Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, 190025, Jammu and Kashmir, India
Mukund Dawale	Department of Plant Pathology and Agricultural Microbiology, PGI, MPKV, Rahuri (M. H.), 413722, India
Neeraj Verma	Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, India
Prachi Singh Baghel	Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, India
Priti Pal	Shri Ramswaroop Memorial Group of Professional Colleges, Lucknow, 226028, India
Pooran Singh Solanki	Birla Institute of Scientific Research, Statue Circle, Jaipur, Rajsthan, India Department of BioEngineering, Birla Institute of Technology, Mesra, Ranchi, Jaipur Campus, Jaipur, Rajasthan, India
P. Ghosh	Birla Institute of Scientific Research, Statue Circle, Jaipur, Rajsthan, India
Pravin Khaire	Department of Plant Pathology and Agricultural Microbiology, PGI, MPKV, Rahuri (M. H.), 413722, India
Poonam Meena	Department of Botany, University of Rajasthan, Jaipur, Raj, India
Prachi Awadhiya	Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, India
Ruchi Gupta	Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, India
Raj Kumar Gothwal	Birla Institute of Scientific Research, Statue Circle, Jaipur, Rajsthan, India Department of BioEngineering, Birla Institute of Technology, Mesra, Ranchi, Jaipur Campus, Jaipur, Rajasthan, India
Rangnath Aher	Department of Botany and Research Centre, New Arts, Commerce and Science College, Parner, Dist- Ahmednagar, 414302, M.S., India
R.K. Naresh	Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut, UP- 250110, India
Rashid Pervez	Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi-11012, India
Sumiah Wani	Plant Virology and Molecular Pathology Lab, Division of Plant Pathology, FoH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, 190025, Jammu and Kashmir, India
S. Ahmad	508 BWT, Eros Garden, Charmwood Village, Faridabad, Haryana, India
Shivani Dubey	Acropolis Institute of Management Studies and Research, Indore, M.P., India

Shamim Akhtar Ansari	Institute of Forest Research and Productivity, Ranchi, 835303, India
Sampat Nehra	Birla Institute of Scientific Research, Statue Circle, Jaipur, Rajsthan, India
Sapana Khandelwal	S.S. Jain Subodh PG Mahila Mahavidyalaya, Jaipur, Raj., India
Sirisha Thakare	Department of Plant Pathology and Agricultural Microbiology, PGI, MPKV, Rahuri (M. H.), 413722, India
Someshree Mane	Department of Plant Pathology and Agricultural Microbiology, PGI, MPKV, Rahuri (M. H.), 413722, India
Sanjay Kolase	Department of Plant Pathology and Agricultural Microbiology, PGI, MPKV, Rahuri (M. H.), 413722, India
Swati Sachdev	Era University, Sarfarz ganj, Lucknow-226003, India
Sumaira Hamid	Department of Biosciences, Integral University, Lucknow, 226026, UP, India
Tapas Biswas	Department of Agricultural Chemistry and Soil Science, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, P.O.-Krishi Viswavidyalaya, Mohanpur, Dist. Nadia, 741252, WB, India
T.C. Archith	Department of Plant Pathology, College of Horticulture, India
V. Devappa	Department of Plant Pathology, College of Horticulture, UHS Campus, G.K.V.K. Post, Bengaluru, 560065, India
Varsha Sharma	Department of Botany, University of Rajasthan, Jaipur, Raj, India
Vandana Gupta	Department of Microbiology, Ram Lal Anand College, University of Delhi, New Delhi, India

CHAPTER 1

An Overview of Mycorrhizae: Nature's Own Biofertilizers

Lalit Mohan[1] and **Vandana Gupta**[1,*]

[1] *Department of Microbiology, Ram Lal Anand College, University of Delhi, 5 Benito Juarez Road, Dhaula Kuan, New Delhi-110021, India*

Abstract: Mycorrhizae are mutualistic associations between plant roots and fungi, conferring several advantages to plants, improving their survival and growth even under harsh soil conditions such as drought, acidic pH, the presence of toxic compounds, low nutrient availability, the presence of soil pathogens, *etc*., and hence act as nature's own biofertilizers. The importance of mycorrhizal associations is signified by the fact that almost all the plant species on our planet carry these associations at least for some part and typically for most of their life cycle. In this chapter, our focus is to provide undergraduate and graduate students with an overview of three different types of mycorrhizae, namely endo-mycorrhizae, ectomycorrhizae, and ectendomycorrizae, based primarily on their macro- and microscopic structures. Further classification of endomycorrhizae into vesicular arbuscular mycorrhizae (VAM), arbuscular mycorrhizae, orchid, and ericoid mycorrhizae and classification of ectendomycorrhizae into monotropoid and arbutoid mycorrhizae are based on further details of microscopic features and the fungal and plant species involved. This chapter also aims at providing the reader with an insight into the advantages conferred by the fungal partner to the plants and the accelerated use of these fungi as inoculants for various applications such as agriculture, afforestation, and reclamation of waste lands.

Keywords: Arbuscular mycorrhiza, Biofertilizer, Ectomycorrhiza, Ectendomycorrhiza, VAM.

INTRODUCTION

Albert Bernard Frankin (1885) discovered a special association between plant roots and microorganisms. For the first time, he introduced the Greek term "mycorrhiza," meaning "fungus roots". He reported that these were widespread on the roots of many woody plants. He also suggested that these mycorrhizae represent a mutualistic association in which the fungus counterpart absorbs minerals from the soil and humus and transfers or translocates them to the

* **Corresponding Author Vandana Gupta:** Department of Microbiology, Ram Lal Anand College, University of Delhi, 5 Benito Juarez Road, Dhaula Kuan, New Delhi-110021, India; E-mail: vandanagupta72@rediffmail.com

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plants, which in turn provide nutrition to the fungus [1]. The discovery by A.B. Frank remained a topic of controversy for almost 40 years, but several related observations and experiments discarded all the controversies, establishing the existence of mycorrhizal associations with a wide range of plants. The existence of mycorrhizae has also been confirmed by fossil records. The records also suggest the evolution of plants along with mycorrhizae [1]. Though Frank was not the first to discover the mycorrhizae (now known as ectomycorrhizae), he is credited because he did his research and his interpretations led him to logical conclusions [2]. Frank was also the first to study in detail the stages of development of ectomycorrhizae, starting from the point of contact of the hypha with the roots to their complete development [1].

The most common fungal partners forming mycorrhizal associations are members of Zygomycetes, Basidiomycetes, and Ascomycetes. There are at least seven different kinds of mycorrhizal associations depending on the type of host plant, the fungus partner, and the nature of interaction between the host plant and the fungal partner.

The term "colony of mycorrhizae" refers to the hyphae of the mycorrhizal fungus originating from one entry point inside the root or one propagule in the soil. The term colonization refers to the extent or degree of the occupation or coverage of the roots by the mycorrhizal fungus. The features of the host plant, the mycorrhizal fungus, as well as the soil and environmental conditions, regulate the mycorrhizal associations (Fig. **1**) [3].

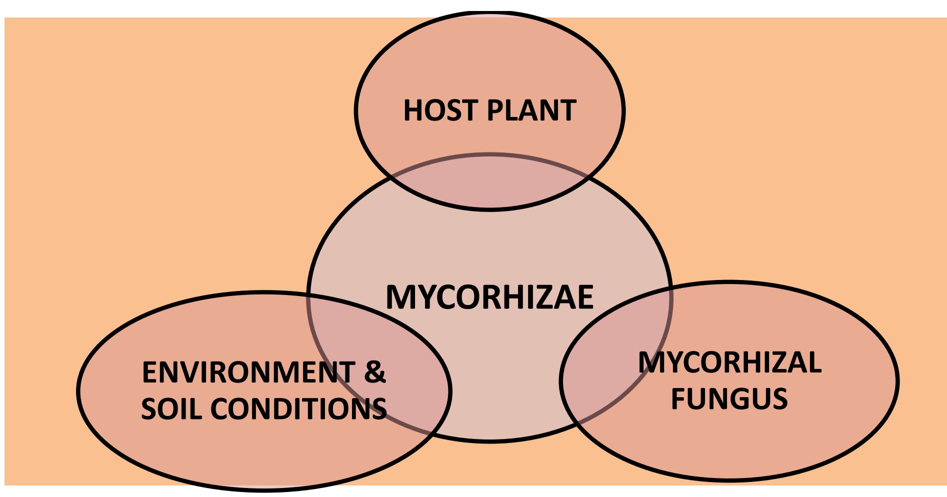

Fig. (1). Three-way interaction involved in the development of the mycorrhizal associations.

The factors influencing the occurrence of the effective mycorrhizal association are: (a) Properties of the roots of the host plant; (b) Climatic factors; (c) Host-fungus compatibility; (d) Disturbances in the soil; and (e) Organisms present in the soil. The mycorrhizal fungi constitute a dominant component of the soil microflora with restricted saprophytic abilities. The effectiveness of mycorrhizal association, such as the amount of soil hyphae produced compared to the root hyphae and physiological characteristics such as nutrient uptake and translocation, is mainly governed by the endophytic properties of the mycorrhizal fungus. Steps in the formation, maturation, and senescence of mycorrhizal associations are depicted in Fig. (**2**).

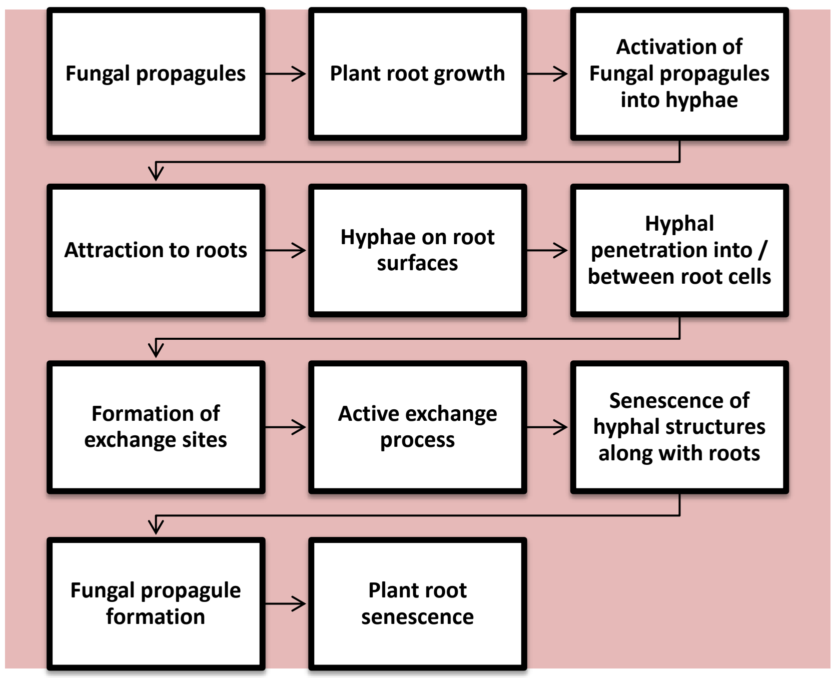

Fig. (2). Sequential steps in the development of mycorrhizal associations, maturation of the association, senescence, and propagule formation.

The mycorrhizal association helps to improve plant productivity and connects the plants to the soil *via* a network of hyphae. The association plays an important role not only in the uptake of various key elements such as nitrogen, phosphorus, iron, calcium, and carbon in the plants, which have lost their photosynthetic capabilities and parasitize mycorrhizal fungi associated with neighbouring photosynthetic plants to fulfil their carbon requirement; approximately 400 plant species form these types of associations. Not only uptake, but mycorrhizal associations also facilitate the solubilization of at least some minerals like phosphorus and iron,

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CHAPTER 2

Mycorrhizal Symbiosis: A Journey from Soil to Commercial Application

Ekta Narwal[1,*], **Amar P. Garg**[2], **Jairam Choudhary**[3] and **R.K. Naresh**[4]

[1] *Department of Agriculture and Agri-informatics, Shobhit Institute of Engineering and Technology, Meerut, UP-250110, India*

[2] *Swami Vivekanand Subharti University, NH-58, Meerut, UP-250005, India*

[3] *Division of Organic Agriculture Systems, ICAR-Indian Institute of Farming System Research, Meerut, UP-250110, India*

[4] *Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut, UP-250110, India*

Abstract: Mycorrhizae are mutualistic symbiotic associations between fungi and plants. Mycorrhizal associations are believed to be established between the Ordovician and Devonian periods. The mycorrhizal association is prevalent in almost all ecosystems with a high degree of host specificity. About 40,000–50,000 fungal species colonize the roots of nearly about 250,000 plant species. These symbiotic relations benefit associated plants by providing up to 80% of N and P and also help in plant growth and fitness by different mechanisms. A look into the recent literature suggests that mycorrhizal fungi are not only involved in improving crop yield but also increase the quality of products through the increase in antioxidants, vitamins, and essential trace elements in plants. Due to eco-friendly and sustainable aspects, widespread research and industrial applications of AM fungi are trending in today's world. During recent years of urbanization and industrialization, the concentration of trace elements has increased in soil and water. Recovery of contaminated areas is very crucial as it may get into the food chain and the process is generally complex. For this, mycorrhizae have evolved as an efficient and sustainable aspect. Ecological restoration of mining sites using AM fungi is considered necessary and useful.

AMF displays significant positive effects, such as increased plant survival under unfavourable growth conditions, enhanced growth and nutrition, improved soil structure and quality, and greater plant re-establishment. Implementation of various molecular techniques and advanced scientific knowledge on AM fungal symbioses, mycorrhizal biotechnology has reached various application domains such as horticulture, agriculture, soil reclamation, bioremediation, gardening, landscaping, and other areas of the plant market.

* **Corresponding author Ekta Narwal:** Department of Agriculture and Agri-informatics, Shobhit Institute of Engineering and Technology, Meerut, UP- 250110, India; E-mail: enarwal@gmail.com

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Keywords: Agriculture, Bioremediation, Biodiversity, Mycorrhiza, Reclamation, Symbiotic association.

INTRODUCTION

Mycorrhiza is a mutual symbiotic association between certain soil fungi and roots of the higher plants. AM symbiosis in plants is approximately 480 Mio years old and it is found in the majority of land plants in most taxa and virtually all ecological niches. Research findings suggest that mycorrhizal associations with plants were established in the Ordovician and Devonian periods [1]. This symbiotic association is known to play a key role in the evolution of land plants in the reducing and harsh environment prevalent at that time [2]. About 50,000 fungal species form mycorrhizal associations with 250,000 plant species. The association between AMF and the roots of plants can be called a facultative symbiosis because colonized plants can benefit from the AM symbiosis but can also live without them. However, some plant species called mycoheterotrophs are known to be obligate parasites on AM fungi because they are completely dependent on AM fungi for their nutrition as they have lost their photosynthetic capacity. On the other side, there are plants, which do not interact with AM fungi, for example, Brassicaceae, Chenopodiaceae, Polygonaceae, Juncaceae, Caryophyllaceae, and Proteaceae, and evolved alternative strategies to meet their nutritional needs [3].

The significant and primary role of mycorrhizal fungi is to provide plant nutrients which are often in limiting conditions [4]. Secondary functions include providing plants protection against soilborne plant pathogens and alleviating heavy metal phytotoxicity, increasing photosynthesis and hormone production, reducing oxidative stress, and providing resistance from biotic and abiotic stresses [5, 6]. Mycorrhizal symbiotic associations provide approximately 80% of nitrogen [N] and phosphorus [P] to the plants and help in the growth and development of associated plants. Mycorrhization also plays a very important role in organizing and maintaining the structure of plant communities in any ecosystem [7] as well as soil microbial communities in the rhizosphere [8]. Mycorrhizal fungi have adaptive homoplasticity in their physiology and metabolism, which makes them more tolerable and adaptive when exposed to stress.

TYPES OF MYCORRHIZA

The mycorrhizal association is present in almost all ecosystems. There are two major groups of mycorrhiza, based on the degree to which hyphae penetrate the root epidermis and its further development: endomycorrhiza and ectomycorrhiza, in both conditions, they penetrate the epidermis and invade plant root cells for nutritional exchange of carbon, nitrogen and phosphorus. Endomycorrhiza forms arbuscules, vesicles and spores inside the host cell that are further categorized into arbuscular mycorrhiza [AM], orchidaceous mycorrhiza and ericoid mycorrhiza, whereas ectomycorrhiza forms Hartig net and thick mantle (Fig. **1**). Some mycorrhizal fungi form both types of mycorrhizal structures called ecto-endomycorrhiza, which are found in the members of subfamily Ericaceae and Arbutoideae. The majority of plants have at least one type of association out of four [7].

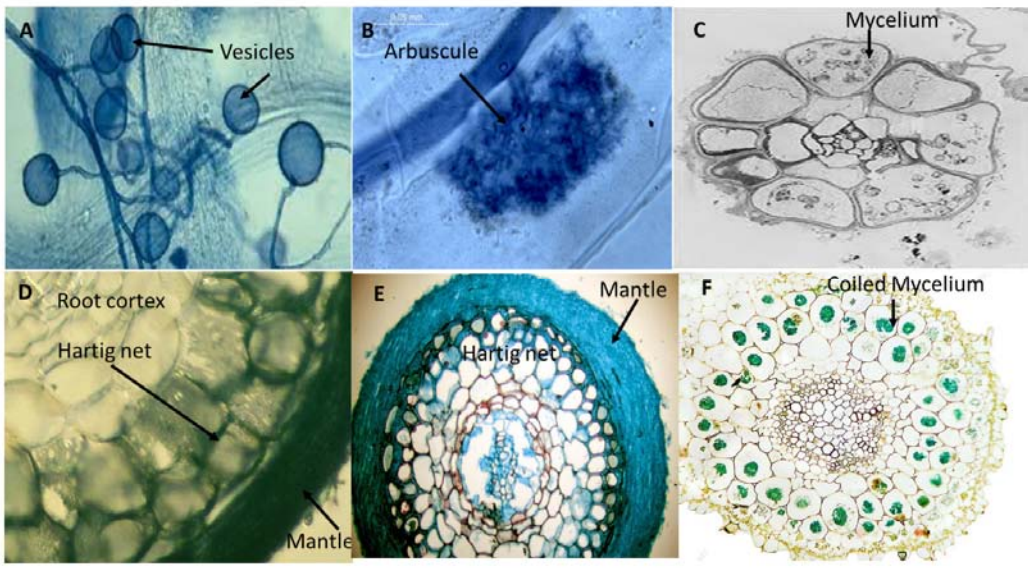

Fig. (1). Types of Mycorrhizal associations in plant roots. (A and B) Arbuscular mycorrhiza showing vesicles and arbuscules inside root cells. (C) Ericoid mycorrhizal colonization in ericoid hair root of *Leucopogon ericoides* (Source: https://mycorrhizas.info). (D) Ectomycorrhizal colonization in roots of *Pinus strobes* (note the heavy mantle outside the root and Hartig net in the intercellular spaces). (E) Cross-section of a root showing mantle and Hartig net and (F) Transverse section through the root of a bird's nest orchid (*Neottia nidus-avis*), showing cells of endotrophic orchid mycorrhiza.

Arbuscular Mycorrhiza

The AM fungal associations are found in all terrestrial ecosystems, including aquatic plants and agroecosystems as well as metal-polluted soils. Around 74% of the total land plants are colonized by AM fungi, of which more than 80% of vascular land plants are associated with AM fungi [9]. In this symbiosis, AM fungi produce hyphae, arbuscules, vesicles, and spores inside the root cortex of the host and hyphae, vesicles, and spores outside the roots. AM fungi are obligate biotrophs, which always need the roots of living hosts to grow and complete their life cycles. There is no synthetic medium for the proliferation of AM fungi *in vitro*.

CHAPTER 3

Arbuscular Mycorrhizal Fungi Association with Plants: Beneficial for Growth, Yield and Stress Management

Prachi Awadhiya[1], **Ruchi Gupta**[1], **Prachi Singh Baghel**[1] and **Neeraj Verma**[1,*]

[1] *Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, 485001 (MP), India*

Abstract: Abiotic pressures interfere with plant growth and production. Climate change and agricultural stress, including the overuse of chemical fertilizers and pesticides, have worsened the effects of abiotic stress on crop yields and damaged ecosystems and their environment. There is an urgent need for environmentally friendly management techniques such as the use of arbuscular mycorrhizal fungi (AMF) to increase crop productivity. AMF is best accepted as a biofertilizer. Additionally, it is broadly believed that the inclusion of AMF provides plant tolerance to a variety of stressful conditions such as temperature, salt, drought, and metals. AMF can provide essential plant nutrients that can hold plants, resulting in enhanced growth and harvest under less stressful and oppressive conditions. The role of AMF as a biofertilizer may improve plant flexibility in a changing environment. Therefore, further research focusing on promoting and producing plant quality produced by AMF is needed. The current review provides an in-depth knowledge of AMF and its impact on plants beyond the various stages of growth and, consequently, the importance of the relationship between different plant nutrients and AMF.

Keywords: Arbuscular mycorrhizal fungi, Abiotic and biotic stresses, Biofertilizer, Growth, Plant growth, Yield.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) enable plants to grow vigorously under stressful conditions by mediating a series of complex interactions between plant and fungus, leading to increased levels of photosynthetic and other factors related to gas exchange [1]. Several reports describe improved resistance to various stresses, including drought, salinity, weeds, temperature, metals, and diseases [2, 3]. About 90% of plant species, including flowering plants, bryophytes, and

* **Corresponding author Neeraj Verma:** Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, 485001 (MP), India; E-mail: neerajverma.2008@yahoo.com

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ferns, can develop AMF-dependent connections [2]. AMF forms vesicles, arbuscules, and hyphae at the plant roots as well as hyphae in the rhizosphere. The interaction of the hyphal network of AMF with plant roots greatly enhances root access to a large area of soil, resulting in improved plant growth [4] and improved plant nutrition by increasing the availability and distribution of various nutrients [5]. AMF also improves soil quality by influencing its composition and texture [6, 7]. Fungal hyphae can accelerate the decay process of the earth's resources [8]. In addition, AMF can affect the decomposition of atmospheric CO_2 by host plants and the movement of photoassimilates from aerial parts to the roots. The current review focuses on the role of AMF as a bio-fertilizer in controlling plant growth and development through improved nutrient access under stressful conditions.

CHARACTERISTICS OF AMF SYMBIOSIS

The symbiotic association of AMF with plants is an ancient example of a cooperative relationship that can control and enhance plant growth and development. A symbiotic relationship between AMF and plants has been reported 400 million years ago [9]. Symbiotic associations are established as a result of various biological processes, leading to several beneficial effects for both [10]. The mycelial network of fungi passing under the roots of the plant promotes the absorption of nutrients that are not otherwise available. Fungal mycelium can surround the roots of several plants, even of different species, and form a common mycorrhizal network (CMN) [11, 12]. AMF colonization enhances the tolerance of stress-induced plants by bringing about several changes in their physiology [13]. AMFs are natural regulators of plant growth and are used as bio-inoculants around the world. Scientists are promoting their use as an eco-friendly biofertilizer for sustainable yields [14].

Growth-related activities of the plants, such as digestion, leaf energy, water-related content (WRC), photosynthesis (especially PSII efficiency), and CO_2 comparisons are affected by the inoculation of AMF [15, 16]. AMF can also modify the upper branches and tissues to improve water tolerance. In addition, the accumulation of dry matter is improved by the inoculation of AMF, leading to improved water absorption and thus increased plant tolerance against stress such as drought and salt. The use of AMF in plant growth in various ecosystems can significantly increase growth and productivity.

AMF as a Bio-Fertilizer

Biofertilizers are plant fertilizers that are made with natural ingredients in order to improve soil fertility. They are very useful for soil health and plant growth [17]. Various research studies conducted on AMF over the past two decades have shown their innumerable benefits to soil health and crop production. Therefore, it is widely believed that AMF can be considered as a non-living fertilizer soon, as mycorrhizal use can effectively reduce the amount of chemical fertilizer application, especially phosphorus [18]. The continued use of organic fertilizers, weed killers, and fungicides has caused various problems for soils, plants, and human health, with detrimental effects on food quality, soil condition, and air and water quality. There is a belief that AMF can reduce the use of chemical fertilizers by up to 50% in good agricultural production, but this figure depends on the type of crop and the stress level.

AMF and Mineral Nutrition

All ecosystems can suffer greatly from excessive land use, which can negatively impact environmental performance as a result [19]. A prominent aspect of such gene transfer relationships is the transfer of organic carbon (C) in the form of lipids and sugars [20, 21]. The colonization of AMF by plants stimulates nutrient uptake. The AMF vaccine can increase the concentration of micro-macro elements and very small molecules, leading to an increase in the production of photosynthate, resulting in increased biomass accumulation [22, 23]. AMF has the potential to increase the absorption of inorganic nutrients in almost all plants, especially phosphate [24].

The AMFs are also effective at absorbing soil nutrients [25]. In addition to macronutrients, AMF is reported to increase the availability of micronutrients such as zinc and copper. The root system's absorption capacity is improved by AMF. AMF tomato plant extracts have shown an increase in leaf area as well as a content of nitrogen, potassium, calcium, and phosphorus levels that indicate improved plant growth [19]. AMF promotes root symbiosis to acquire essential nutrients in the host plant and, as a result, provides mineral salts by restoring, for example, N, P, K, Ca, Zn, and S within root cells. AMF produces fungal formations called arbuscules, which help to exchange rare minerals and compounds of carbon and phosphorus, ultimately providing greater plant handling potential [26, 27]. Therefore, they can significantly increase the concentration of phosphorus in the roots and shoots [28]. Under phosphorus-restricted conditions, mycorrhizal organization enhances the supply of phosphorus to plant-infected roots. For example, the value of Pi detection has improved significantly in maize plants with AMF colonies [29]. Increasing photosynthetic activity and other leaf activities are directly related to the increased frequency of AMF acquisitions, which are directly linked to the acquisition of N, P, and carbon, which go to the roots and enhance the development of root crops. The concentration of P and N and the ratio of N:P in shoots are significantly affected [30]. The content of nitrogen in plant extracts has been reported to significantly change when traditional AMF treatment is utilized [31]. It is widely accepted that molds can

CHAPTER 4

Mycorrhiza: Prospects, Possibilities, and Potential

Shivani Dubey[1,*] and **Mukul Sharma**[2]

[1] *Acropolis Institute of Management Studies and Research, Indore, M.P., India*

[2] *Sri Aurobindo Institute of Pharmacy, Indore (M.P.), India*

Abstract: Mycorrhiza is the mutually beneficial relationship between a plant and a fungus colonizing its roots wherein the plant provides carbohydrates to fungi, in return Mycorrhizal fungal partner imparts several advantages to plants *viz.*, making otherwise unavailable nutrients available to the plant, imparting resistance to diseases. It also has the potential to be used extensively not only for growth and yield but also for disease and insect control along with nutrient cycling, heavy metal assimilation, land reclamation, restoration and so on. The application of mycorrhizal biotechnology has great potential and can play an essential role in the restoration of degraded lands in many surface-mined areas. This chapter is an overview of the prospects, potential and possibilities of Mycorrhiza in agriculture, industry and other environmental applications.

Keywords: Classification, Ectotrophic, Endotrophic, Importance, Mycorrhiza.

INTRODUCTION

Albert Bernard Frank in 1885, introduced the Greek term Mycorrhiza during his study on soil microbe-plant relationship, which literally means fungus-root (Frank, 1885). Mycorrhiza (plural: mycorrhizae), as the name suggests, is the mutually beneficial relationship between plant and fungus colonizing its roots and extending it far into the soil. Mycorrhizal fungal partner effectively absorbs nutrients and water than the roots alone. Associations between plants and symbiotic fungi—mycorrhiza are ubiquitous in plant communities. Studies have revealed that about 95 percent of plant species and more than 150 species of mycorrhizal fungi around the world in all types of soils and climates are involved in such symbiotic relationships. Mycorrhizal fungi colonize environments like alpine and boreal zones, tropical forests, grasslands and croplands [1].

[*] **Corresponding author Shivani Dubey:** Acropolis Institute of Management Studies and Research, Indore, M.P., India; E-mail: shivanidubeysh@gmail.com

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This symbiosis initiates as the fungal spores germinate and emerge as threadlike structures, the hyphae, which enter the epidermis of plant roots and colonize, thereby extending a vast network of hyphae throughout the soil to form a significantly improved absorptive surface area. This results in improved nutrient procurement and uptake by plant roots, particularly elemental phosphorus (P), zinc (Zn), manganese (Mn) and copper (Cu) and water. In return, the plant provides carbohydrates for the fungi through the process of photosynthesis. This relationship is a significant aspect in nutrient cycling, ecology, evolution, and physiology of plants.

CLASSIFICATION OF MYCORRHIZA

Mycorrhiza is conventionally classified into two types on the basis of the location of the fungal hyphae in relation to the root tissues of the plant: ectotrophic and endotrophic where *ecto* means outside the root, *endo* means inside. The main characteristics are given in Table **1**.

Table 1. The characteristics of seven types of mycorrhizae [3].

Features	Endomycorrhizae					Ectomycorrhizas	
	AM	Ericoid	Arbutoid	Mono-tropoid	Orchid	Ecto-	Ectendo-
Fungi septate	No	Yes	Yes	Yes	Yes	Yes	Yes
Intracellular colonization	Yes	Yes	Yes	Yes	Yes	No	Yes
Fungal sheath	No	No	Yes or No	Yes	No	Yes	Yes or No
Hartig net	No	No	Yes	Yes	No	Yes	Yes
Vesicles	Yes or No	No	No	No	No	No	No
Plant host chlorophyllous*	Yes (? No)	Yes	Yes	No	No*	Yes	Yes
Fungal taxa	Glomeromycota	Ascomycota	Basidiomycota	Basidiomycota	Basidiomycota	Basidiomycota and Ascomycota	Basidiomycota and Ascomycota
Plant taxa†	Bryo, Pterido, Gymno, and Angio	Ericales and Bryo	Ericales	Monotropaceae	Orchidaceae	Gymno and Angio	Gymno and Angio

*All orchids are achlorophyllous in the early seedling stages, but usually chlorophyllous as adults.
†Bryo = Bryophyta, Pterido = Pteridophyta, Gymno = Gymnospermae, Angio = Angiospermae.

Ectomycorrhiza

Ectomycorrhiza forms a compact structure of hyphae on the external face of plant roots, but doesn't access the plant root cells. Still, hyphal strands access the root face and grow between cortical root cells and also extend outward from the mantle to the soil face. Ectomycorrhiza generally occurs on pine and most other conifers, birch, beech and oak belonging to Pinaceae, Betulaceae and Fagaceae families independently and other woody plants. Due to their host range, ectomycorrhiza only gives benefits to forestry seedlings and woody ornamentals.

Ectomycorrhizas are the most advanced symbiotic association between advanced plants and fungi, involving about three seed plants including the majority of timber trees (Fig. **1**). In this association, the plant root system is fully girdled by a jacket of fungal tissue, which can be more than 100 µm thick, though it is generally over to 50 µm thick. The hyphae access between the outmost cell layers forming the Hartig net. From this, a network of hyphal rudiments (hyphae, beaches and rhizomorphs) extends out to explore the soil sphere and affiliate with the fungal tissue of the root. Ectomycorrhizal fungi are substantially Basidiomycota and include common forest land mushrooms, similar to *Amanita* spp., *Boletus* spp. and *Tricholoma* spp. Ectomycorrhizas can connect together groups of trees. Ectomycorrhizal fungi depend on the plant host for carbon sources, the utmost being uncompetitive as saprotrophs. With many exceptions (like *Tricholoma fumosum*), the fungi are unfit to use cellulose and lignin; but the fungus provides greatly enhanced mineral ion uptake for the plant and the fungus is suitable to capture nutrients, particularly phosphate and ammonium ions, which the root can not pierce. Host shops grow inadequately when they warrant ectomycorrhizas (Fig. **1**). This ectomycorrhizal group is nicely homogenous, but a group, ectEndomycorrhizae, has been added.

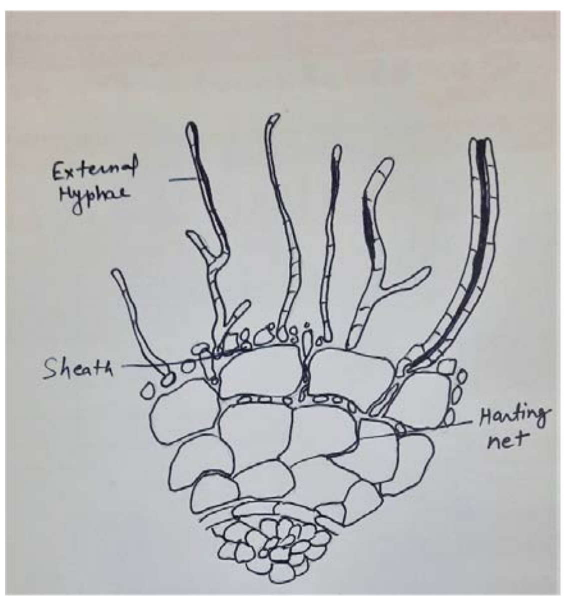

Fig. (1). Ectomycorrhizae.

CHAPTER 5

Agricultural Application of Mycorrhiza on Growth, Yield, and Quality of *Lycopersicon Esculentum* Mill-A Case Study

Rangnath Aher[1,*]

[1] *Department of Botany and Research Centre, New Arts, Commerce and Science College, Parner, Dist- Ahmednagar, 414 302, M.S., India*

Abstract: The present investigation deals with the effect of six different species of mycorrhizal inoculation on the germination and growth of *Lycopersicon esculentum* Mill (Tomato). This experiment was conducted to observe the efficient VAM inoculation that would be beneficial for plant growth. Tomato occupies a prominent position in vegetables and is a commercially exploited crop. The local variety of tomatoes (1057) was inoculated with six different AM fungal inoculums in the germinating media. The germination percentage and plant vigour were increased by different VA mycorrhizal fungi. The minimum number of days taken for germination was observed by *Glomus fasciculatum (*6 days) followed by *G. mosseae, G. monosporum, G. heterosporum, G. geosporum* and *G. multicaule* (7 days). The highest germination percent was recorded with *G. fasciculatum* (96%) followed by *G. geosporum* (94.12 per cent) when compared to the control. The highest shoot height, root length, fresh shoot weight and the highest fresh root weight were recorded with *Glomus fasciculatum* compared to the control and other VA fungal species. Hence, it is concluded that AM fungi help in better seed germination by mutualistic symbiosis.

Keywords: Germination, *Glomus* spp., Mycorrhiza, Mutualism, *Lycopersicon esculentum*.

INTRODUCTION

The present investigation deals with the effect of six various species of mycorrhizal inoculum on the germination and growth of *Lycopersicon esculentum* Mill. This study was conducted to observe the efficient mycorrhizal inoculation that would be beneficial for plant growth. *L. esculentum* occupies a prominent position in vegetables and is a commercially exploited crop. Mycorrhiza are symbiotic associations between plant roots and certain soil fungi that play a key

* **Corresponding author Rangnath Aher:** Department of Botany and Research Centre, New Arts, Commerce and Science College, Parner, Dist-Ahmednagar, 414 302, M.S., India; E-mail: rkaher69@gmail.com

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role in nutrient cycling in the ecosystem and also protect plants against environmental and cultural stress. Most of the major plant families are able to form mycorrhiza. The arbuscular mycorrhizal (AM) association is the commonest mycorrhizal type involved in agricultural systems.

Frank [1] described mycorrhiza as a mutualistic association of plant roots and fungi for which the term “Mycorrhiza” he has coined. The involvement in mineral uptake from the soil, they referred to as 'phosphorus gathering fungi'. Mycorrhizal fungi increase the surface absorbing area of roots, thereby greatly improving the ability of the plant to access various soil resources. Mycorrhizae are an essential part of a healthy soil microbiome and several miles of fungal filaments can be present in less than a thimbleful of soil is very significant.

L. esculentum Mill. is the edible, often red fruit/berry of a commonly known tomato plants all over the world. The species originated in the South American Andes. The tomato is useful in various ways, including raw, as an ingredient in many dishes, sauces, salads, drinks and vegetables. The fruit is rich in lycopene, which may have beneficial health effects. The tomato belongs to the family Solanaceae. The plants typically grow to 1–2.5 meters (3–9 ft) in height and have a weak stem that often sprawls over the ground and vines over other plants. It is a perennial in its native habitat, although often grown outdoors in temperate climates as annual.

Importance of the mycorrhizal fungi has received considerable attention in recent years owing to their beneficial response in improving crop productivity in agriculture and forestry. Mycorrhizal fungi increase nutrient uptake not only by increasing the surface absorbing area of the roots, but also release powerful organic compounds into the soil that help to solubilize hard-to- capture nutrients, such as organic nitrogen, phosphorus, iron and many other “tightly bound” soil nutrients. This extraction process is particularly important in plant nutrition and explains why non-mycorrhizal plants require high levels of fertilizers to maintain their health. Mycorrhizal fungi form an intricate web that captures and assimilates nutrients, conserving the nutrient capital in soils [2 - 6].

MATERIALS AND METHODS

The present investigation was carried out as a pot culture technique in Post Graduate Department of Botany, New Arts, Commerce and Science College, Parner, Maharashtra during the period 2012-20163. The local variety of tomato (1057) seeds used for the experiment was collected from the local market. The seeds were sown in plastic pots with six VAM species and a control. Cultures of six VAM fungi spores *i.e*. *Glomus fasciculatum*, *G. monosporum*, *G. mosseae*, *G. geosporum*, *G. heterosporum*, and *G. multicaule* were extracted from rhizospheric soil of tomato using wet-sieving and decanting method [7].

Identification of the AM fungi was carried out using relevant literature [8, 9]. The inoculums were multiplied in a sterilized potting mixture using *Zea mays* as a host plant in the shade house in college. After the proper development of inoculums, 4 grams of inoculum treatment was given to all pots except the control.

RESULTS AND DISCUSSION

After a long experimental study, the finding of the present investigation brings out clearly indicated that *Lycopersicon esculentum* Mill responds well to mycorrhizal inoculation under pot culture. During the present finding seedling growth and vigor of tomatoes raised in pots were evaluated after inoculating nursery soil with six various cultures of mycorrhizal fungi. Six mycorrhizal fungi were tested for their ability to increase the growth, and biomass by the colonization of roots. Among the *Glomus fasciculatum*, *G. monosporum*, *G. mosseae*, *G. heterosporum*, *G. geosporum*, and *G. multicaule* mycorrhizal fungi. Among all, *G. fasciculatum* was the most effective in increasing the number of leaves, shoot and root growth, fresh weight, and % infection over control. The germination percentage and plant vigour were tested and it was increased by different VA mycorrhizal fungi. The minimum number of days taken for germination was observed by *G. fasciculatum (*6 days) followed by *G. mosseae*, *G. monosporum*, *G. heterosporum*, *G. multicaule* (7 days) and *G. geosporum* (8 days). The highest germination percent was recorded with *G. fasciculatum* (97%) followed by *G. geosporum* (94.12 per cent) when compared to the control. The highest number of leaves (15.28) compared with control (14.18), shoot height (22.38 cm), root length (13.78 cm), the highest fresh root weight (2 mg), and percent colonization (72%) were recorded with *G. fasciculatum* compared to the control *i.e*. fresh shoot weight (2.28 mg), shoot height (18.10 cm), root length (12.00 cm), fresh root weight (1.08 mg) and shoot weight (1.26 mg) and other VA fungal species. Hence mycorrhizal fungi help in better seed germination by mutualistic symbiosis.

Prasad and coworkers [10] observed the distribution of AM fungi in soybean. The treatment of GF showed a good effect on shoot and root lengths, compared to the control. This is because of the ability of mycorrhizal plants to utilize the available nutrients more efficiently than the non-mycorrhizal plants and mycorrhizal fungi are known to control the root topology in response to soil conditions [11].

A positive influence of VAM on vegetative and reproductive parameters of *Dolichos lablab* and positive response of VAM species on growth, yield and quality of two marigold varieties have been observed [12, 13]. Among the various 12 VAM culture *Gigaspora margarita* showed the most positive response in seed germination, highest shoot height, root length, highest number of roots, and

CHAPTER 6

Wonders of Microbial Community in Modern Industry

Tapas Biswas[1,*]

[1] *Department of Agricultural Chemistry and Soil Science, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, P.O.-Krishi viswavidyalaya, Mohanpur, Dist.–Nadia-741252, WB, India*

Abstract: The existence of microorganisms in nature has been under speculation since ancient times, and they were exploited for beer and vinegar production long before anything was known about their existence. The scientific study of microorganisms began with their observation under the microscope in the 1670s by Antony van Leeuwenhoek. Louis Pasteur, in 1860, reported the first synthetic medium for microorganisms and introduced the biological concept of alcohol fermentation. The next phase started with the use of modern industrial fermentation, aiming for large-scale aerobic fermentation facilities. Selected strains of yeast, *Saccharomyces cerevisiae*, are commonly used for the fermentation of commercial alcohols, namely wine, beer, and distilled liquor. Vinegar is prepared by allowing a wine to go sour with the aid of a specific microbe under controlled conditions. Cider vinegar is made from alcohol in fermented apple cider, whereas wine vinegar comes from grapes. Genetic alteration of microorganisms has been an important practice in many industries, including agriculture, the beverage industry, the pharmaceutical industry, *etc*. The prerequisites to a practical industrial microbiological process are the organisms, medium, and product upon which the whole gamut of production depends. The discovery of penicillin by Alexander Fleming in 1929 triggered an intensive search for antibiotics during the Second World War, and several other antibiotics were discovered. The wonderful activities of the microbial community are now exploited by industrial microbiologists to find suitable microorganisms for desired products such as antibiotics, amino acids, food products, enzymes, amino acids, vaccines, organic solvents, and other value-added products. The benefits of microbial activities are also widened commercially in other fields, like the agriculture sector, through biofertilizer and biopesticide preparations. Carrier based bio-inoculants are agriculturally useful in terms of nitrogen fixation, phosphorus solubilization, or nutrient mobilization, to increase the productivity of soil and crop. Most commercial biopesticides are of microbial origin and are primarily based on the *Bacillus thuringiensis* (Bt) microorganism. Potential microorganisms are exploited in many other sectors, from petroleum, mining, textiles, polymers, cosmetics, waste treatment, health care, and so on. Industrial production of citric acid is also accomplished by microbial fermentation using the fungus. Many microorganisms are capable of synthesizing bioactive L-optical

[*] **Corresponding author Tapas Biswas:** Department of Agricultural Chemistry and Soil Science, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, P.O.-Krishi Viswavidyalaya, Mohanpur, Dist.–Nadia-741252, WB, India; E-mail: tapas.acss@gmail.com

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isomers of amino acids from inorganic nitrogen compounds. Commercially useful enzymes are manufactured from microorganisms using 'immobilized enzyme technology'. Among the commercially available enzymes, proteases and amylases are produced in maximum quantities. Insulin is another very important pharmaceutical product, produced commercially by a genetically engineered bacteria. Recombinant (r-) DNA technology has been exploited in order to provide selective improvements in various specialties that include crop agriculture, pharmaceutics, gene therapy, vaccine design, and bioremediation. The technology has now become the mainstay of the pharmaceutical industry. Natural genetic engineering uses 'forced evolution' and 'adaptive mutation'. Such 'environmentally directed mutation' can produce microbes with new biosynthetic capabilities. Extremozymes from extremophiles are becoming increasingly attractive as biocatalysts for industrial applications, particularly at high temperatures. However, a vast microbial world is yet to be examined for its efficacy towards new industrial products. So, research on industrially useful microorganisms has tremendous potential and a long way to go.

Keywords: Antibiotic, Amino acid, Alcohol, Biofertilizer, Enzyme, Fermentation industry, Microorganisms, Recombinant (r-) DNA technology, Yeast.

INTRODUCTION

An industry can be defined as an organization of economic activity concerned with the manufacture, extraction, and processing of raw materials, or construction. The microbial world, although mostly invisible, can be an active player in the processing of raw materials and manufacturing products by using machinery and factories. The use of microbes on an industrial scale is an economic activity as well. Industrial microbiology is a branch of applied microbiology in which microorganisms are used for the production of important substances such as antibiotics, amino acids, food products, enzymes, vaccines, and fine chemicals. Microorganisms were exploited for development long before anything was known about their presence. As early as 6000 BC, the Babylonians and Sumerians used yeast to make alcohol. History reveals many other applications of a microbiological process that resulted in the production of desirable materials, particularly foods and beverages. However, it was not until the studies of Louis Pasteur in the second half of the nineteenth century that the role of microorganisms in this process was understood. Alcohol was probably the first product of ancient biotechnology. It has now become possible for us to discover or improve better strains of microbes for commercial production. Under natural conditions, microorganisms produce a large variety of chemical substances. Some of these substances are useful in disease treatment, while others are valuable raw materials for different industries. The activities of the wonderful microbial community are exploited on a large scale to produce chemical products and carry out important chemical transformations. The task of industrial microbiologists is to find suitable microorganisms for use in modern industry and get the desired product. Different highly efficient microorganisms are presently being used for the production of a wide range of products at an industrial scale. A wide range of approaches, starting from isolating microorganisms from nature, mostly soil, to using sophisticated molecular techniques to modify an existing microorganism [1] and make it suitable for microbial application, have been embraced on a large scale. In biotechnology and biomanufacturing, these tiny microbes stand like miniature chemical factories that produce vital products such as amino acids, enzymes, medicines, and food additives [2]. Modern biotechnology involves the use of recombinant DNA technology, enzyme engineering, genetic engineering practices, *etc*., for newer products. The application of biotechnology in mining also gained attention, where microorganisms are used to leach metals from low-grade ores. Microbes can also degrade waste to reduce industrial pollution. For the benefit of agriculture, industrial production of biopesticides and biofertilizers is now contributing tremendously towards the control of insect pests and the enhancement of plant growth, respectively.

The utility of microorganisms in the industry can be categorised into three major phases. In the first phase, traditional industrial microbiology involved the application of microorganisms to preserve milk and vegetables and produce food products such as cheese, bread, pickles, beer, wine, and vinegar. The second major phase started with the use of modern industrial fermentation, guiding large-scale fermentation facilities to process various enzymes, vitamins, organic solvents, antibiotics, and other value-added products. Microbiological engineering came into being through the development and production of the antibiotics penicillin and streptomycin. The third phase is modern microbial biotechnology using microbial recombinant DNA technology. It has a wide spectrum of industrial applications ranging from agriculture, food processing, detergents, dairy, beverages, the paper industry, leather, petroleum, mining, textiles, polymers, cosmetics, waste treatment, and health care to diagnostics, pharmaceuticals, and medicine. The recent COVID-19 pandemic crisis offers opportunities to foster industrialization by strategically building synergies [3, 4] between policy areas such as healthcare and industry [5]. Health-related research and development (R&D) drives economic dynamism through pharmaceutical and related industries and social wellbeing *via* vaccine and medicine development. Pharmaceuticals are among the most research-intensive activities in Organisation for Economic Co-operation and Development (OECD) member countries, which still account for one of the largest shares of global R&D expenditure. Under the current global pandemic situation, the demand for generic drugs and their production have increased. India's strategy has been to tap into external demand to boost the local pharmaceutical industry's development. In the post-COVID-19 world, India and other countries with advanced productive and innovation capacities have the opportunity to continue tapping into the growing demand in

CHAPTER 7

Soil Microbes as a Tool for Industry and Research

Priti Pal[1], **Humaira Rani**[2], **Shamim Akhtar Ansari**[3], **Swati Sachdev**[4], **Abdul Raheem**[5], **Mohammad Amir**[5] and **Mohammad Israil Ansari**[5,*]

[1] *Shri Ramswaroop Memorial Group of Professional Colleges, Lucknow-226028, India*

[2] *Lotus Institute of Pharmacy, Bareilly-243123, India*

[3] *Institute of Forest Research and Productivity, Ranchi-835 303, India*

[4] *Era University, Sarfarz ganj, Lucknow-226003, India*

[5] *Department of Botany, University of Lucknow, Lucknow-226 007, India*

Abstract: Soils are a rich source of biologically active industrial and medical compounds arising from microbial populations and their ecosystem services that comprise soil microbiome. The research of soil microbial ecosystems has supported the development of a complete knowledge of the earth's microbial community's (bacteria, archaea, lower and higher eukaryotes, and viruses) important role in repairing soil structure and function and making it active. Soil microbiome discovery has transformed environmental problems, agricultural productivity, bio-manufacturing and medical science. Soil microbes are an obligatory fundamental form of life affecting us in a variety of ways, helping as tools in industry and research. Soil microorganisms in the biosphere play a crucial role in supporting life in the face of increasing 21st century challenges such as soil fertility, food insecurity, epidemics, and a global energy dilemma.

Algae, fungi, mushrooms, protozoa, seaweeds, and, in particular, soil microorganisms now represent an unlimited source and ingredients used in pharmaceuticals for the manufacture of antibiotic compounds, in food industries for the advancement of human nutrition, in medication and beauty care products, in climate control, in the industry for the creation of fuel, chemicals, and other bioactive mixtures, and in research. A detailed knowledge of soil microorganism resilience might lead to new advances in agriculture, energy, healthcare, and the environment.

Keywords: Bioactive compounds, Remediation, Soil microbiomes.

[*] **Corresponding author Mohammad Israil Ansari:** Department of Botany, University of Lucknow, Lucknow-226 007, India; E-mail: ansari_mi@lkouniv.ac.in

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INTRODUCTION

Soil, the terrestrial planet's outermost part, is one of the most essential natural resources and vital components of the ecosystem. Soil provides a variety of ecosystem services such as food production, climate and water cycle management, energy generation, and habitat for many living things. This significant natural resource of the ecosystem is a site of varied ranges of bacteria (beneficial and pathogenic) known as the soil microbiome, which is defined by archaea and eukarya. The variability of soil microbiomes depends on their collective existence in geographical locations. They can be found in both bulk soil and root-affected soil [1]. Soil microorganisms can also be found in a variety of severe conditions. *Rhizobium*, *Achromobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Serratia*, *Staphylococcus*, *Burkholderia*, *Flavobacterium*, *Exiguobacterium*, *Herbaspirillum*, *Arthrobacter*, *Methylobacterium*, *Paenibacillus*, and *Pseudomonas* have all been discovered to be prevalent as soil microorganisms [2]. The various groups of microorganisms found in soil play a variety of important roles, including soil fertility maintenance, nutrient cycling, organic matter decomposition and fossil fuel formation, and groundwater recycling, which have applications in agriculture as a plant preserver and improved performance [3]. Soil microorganisms can likewise be utilized for the remediation and decomposition of soil contaminations.

Ranchers and scientists are attempting to make the organisms as pests control specialists. In biotechnology and bio-manufacturing, these little organisms and living cells are gainfully employed in pharmaceutical and nutraceutical industries for manufacturing products such as amino acids, enzymes, pharmaceuticals, and food additives. Generally, soil microorganisms are being used to take out fermentation processes, and for millennia, humans have used yeasts, moulds, and bacteria to produce a wide range of food stuff such as yogurt, cheese, vinegar, beer, wine, and bread. By using soil microbes, one can make fermented fish, meat, and vegetables. Microbes are also used in fermenting several kinds of food to produce a wide range of oriental dietary items.

BENEFICIAL SOIL MICROBIOME BIODIVERSITY

Soil bacteria are friendly to many organic species, including animals, mankind, and plants. These tiny organisms are classified as archaea, bacteria, fungi, algae, and protozoans and may be found in a variety of habitats that are always striving to benefit the soil. Soil is one of the earth's ecosystems that support a wide range of microbial diversity from all three domains, namely archaea, bacteria, and eukarya [4]. The study of microbial diversity and the role of organisms to sustainable progress are becoming more extensive by the day. Microbial diversity varies with soil type and environmental factors such as temperature. The texture of the soil, as well as the presence of heavy metals and salt, affect soil bacteria both qualitative as well as quantitative. Microbe heterogeneity varies among soil organizations, such as rhizospheric soil (soil surrounding plant roots) and non-associated soil, such as the bulk rhizosphere, the restricted zone connected with plant roots, and the soil of this area [5]. This soil is home to many different types of microorganisms. Over here, plant roots exude a variety of combinations known as root exudates, which include sugars, natural acids, minerals, chemicals, amino acids, growth hormones, unsaturated fats, and antibacterial substances. These chemicals are being utilized as a source of energy by many soil microorganisms. An enormous variety of microorganisms, including bacteria and fungus, have been recorded from this soil, with the majority of bacteria belonging to the genera *Bacillus* and *Pseudomonas*. Fungal species including *Penicillium* sp., *Trichoderma* sp., *Aspergillus awamori*, *A. fumigates*, *A. parasiticus*, *A. terreus*, *A. luchuensis*, *Chrysonilia sitophila*, *Dothideomycetes* sp., *Eupenicillium* spp., and *Galactomyces candidum* have also been identified from the soil [6 - 8].

The bulk soil is the soil that has not been influenced by any plant. A wide range of soil microorganisms, including *Enterobacter aerogenes*, *Enterobacter asburiae* [9], *Burkholderia* sp., *Klebsiella* sp., *Serratia* sp. [10], *Pseudomonas* sp. [11], *Enterobacter cloacae*, *Pantoea agglomerans*, *Agrobacterium tumefaciens* [12], *Promicromonospora iranensis* sp. nov., *Trichoderma* sp. [13], *Bacillus megaterium*, *Penicillium* sp. [14], *Blastococcus* sp., *Gaiella* sp., *Rubrobacter* sp. [15], *Metarhizium* sp. [16], *Rhizomucor pusillus* [17], *Fusarium proliferatum* [18], and *Methanocella arvoryzae* sp. nov are found in this soil type. Extreme conditions where soil microbial diversity is being examined include saline soil, heavy metals dirt, cold environments, acidic soil, and alkaline soil.

SOIL MICROBIOMES FOR SUSTAINABLE AGRICULTURE INDUSTRY

In the current agriculture industry, along with transgenic crops, high-yielding cultivars, artificial fertilizers, and constant irrigation, the use of soil microorganisms as organic manure has also set a trend. Chemical fertilizers are becoming more economical to employ in agriculture due to their negative effects and high cost [19]. The agricultural efficiency of soil can likewise be developed by organisms found in the soil. Nowadays, naturally found soil bacteria that create biological products with the ability to recycle nutrients and are environmentally beneficial have a wide range of applications (Fig. **1**).

CHAPTER 8

Commercial Exploitation of Various Microbes in Agriculture

C.G. Sangeetha[1,*], **V. Devappa**[1] and **T.C. Archith**[1]

[1] *Department of Plant Pathology, College of Horticulture, UHS Campus, G.K.V.K Post, Bengaluru-560065, 560065, India*

Abstract: The major global challenge in the present scenario is to provide nutritional security to the growing population without affecting the ecosystem or the environment. Crop productivity mainly suffers because of various pests, diseases, and other problems caused by the use of pesticides during the management. Pesticide residue is now being considered as more detrimental to human health. Hence to overcome these biotic problems, the use of biological organisms such as arbuscular mycorrhizal fungi (AMF), *Trichoderma* spp., plant growth-promoting rhizobacteria (PGPR) and endophytes are gaining popularity to achieve sustainable agriculture. Still, many microorganisms should be identified in order to know their ecological significance. The proper selection and the application of the microbes have huge potential to safeguard our food and environment. Furthermore, novel and modern techniques like clustered regularly interspaced short palindromic repeats (CRISPR/Cas), transcriptomics, proteomics, genomics, *etc*., can be exploited for the sustainability of the crop ecosystem. The microorganisms can be improved by gene engineering techniques, which will improve the overall health of the plants. Thus, this chapter presents a brief overview of recent trends in the application of various microbial interactions with the twenty-first century technology for crop productivity and the overall sustainability of our agricultural ecosystem for our future generation.

Keywords: Bio-priming, Endophytes, Mycoherbicides, Microorganisms, Rhizosphere, Secondary metabolites.

INTRODUCTION

The world's population is expected to reach 9.6 billion by the end of 2050, which has been estimated as maximum capacity on planet [1]. To meet the increasing population, food production has to be doubled, which is a very challenging job with the decline in the cultivable land [2]. In addition, another major challenge would be to ensure the availability of nutritional and safe food. The crop produc-

* **Corresponding author C.G. Sangeetha:** Department of Plant Pathology, College of Horticulture, UHS Campus, G.K.V.K Post, Bengaluru-560065, India; E-mail: sangeethacg@gmail.com

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tivity should be increased to meet the increasing demand for food along with maintaining the overall plant health. Currently, to optimize food production, farmers have been indiscriminately using chemicals [3]. The indiscriminate use of these chemicals is reported to cause a lot of hazards to our various ecosystems [4, 5]. In this regard, the present priority is to reduce the use of inorganic fertilizers, maintain the productivity of crops, and use novel innovations in crop productivity with limited resources to feed the increasing population. India has achieved self-sufficiency in food grain production but not in achieving sustainability. Hence there is a need for improving the production of replacements for chemicals that have the ability to enhance productivity in addition to improving plant nutrition, stress tolerance and protection from diseases and insect pests to meet the growing demand [6]. Although the number of biocontrol products in plant disease management is increasing, still these products represent only 1% of the agricultural control measures while fungicides account for 15% of total chemicals used in agriculture. In recent years many small and large entrepreneurs have entered into the commercial production of bio-control agents resulting in the entry of various biocontrol products into the world market. In this scenario, the use of microbial inoculants will be the effective option without further deteriorating the existing resources or ecosystems for the coming generations [5, 7, 8].

MYCOHERBICIDES

The application of biological control for weed management has been generally viewed as an ecologically friendly approach. It has been more than a decade since the first fungal herbicides were commercialized by DeVine and Collego, which were the first registered mycoherbicides consisting of a suspension of chlamydospores of *Phytophthora palmivora* to control *Morrenia odorata* [9]. A mycoherbicide is a fungal strain that is pathogenic for one or more weeds. Mycoherbicides are generally applied as a spray to young weed plants within a crop. Few *Trichoderma* spp. have been reported to express mycoherbicide property. About fifteen *T. harzianum* strains, which show the effect on the weed, have been mentioned [10]. Treating the soil and seeds can help in inhibiting the growth and emergence of weeds. *T. virens* produces an antibiotic called viridiol, a phytotoxin, which affects the germination of two weeds *Setaria viridis* and *Amaranthus retroflexus* [11]. The culture filtrate of *T. pseudokoningii*, *T. reesei*, *T. harzianum*, and *T. viride* exhibited high inhibition of seeds and seedlings of *Parthenium hysterophorus* L. (weed) [12]. The culture filtrate of four *Trichoderma* species such as *T. harzianum*, *T. pseudokoningii*, *T. reesei*, and *T. viride* extracted by using the solvent n-hexane and ethyl acetate when sprayed on the leaf of wheat weeds *Rumex dentatus* L. caused high toxicity [13]. The mycelium extract of *T. harzianum* showed toxicity on *Lemna minor* (Duck weed) [14]. The extract of *T. koningiopsis* contained several effect enzymes including amylases, peroxidases, lipases and cellulases, which were toxic to the weed *Euphorbia heterophylla* [15]. The potential bioherbicide commercializing agents has often been dependent on the ease and economics of mass producing and formulating large amounts of viable, stable, and highly efficacious microbial propagules [16]. However, mycoherbicides have not been largely applied in field and horticultural crops for weed management because it requires specific humidity conditions which determine their effectiveness as compared to chemicals. In the future, biotechnological advances will reverse this situation and improve the performance of mycoherbicides.

PLANT-MICROBE INTERACTION

Rhizosphere

The rhizosphere contains many microorganisms that inhabit above or under the ground, which possess diversity and are beneficial for the growth and development of plants. The root zone of the plant in the rhizosphere has a wide range of microorganisms such as plant growth-promoting rhizobacteria (PGPR), *Rhizobium*, *Trichoderma* spp., endophytes, and arbuscular mycorrhizal fungi (AMF). The balance established among the microbes and plants promotes better disease-free growth and development. Therefore, the microbiome associated with plant plays an important role in the nutrition of the plant, growth, and different cycles of carbon or nitrogen and also during biotic or abiotic stress conditions [17, 18]. In horticultural crop ecosystems, the productivity is largely affected by the interactions among the plants and the microorganisms [19]. The microbial community is very distinct for each rhizosphere [20]. The soil microbe's interaction and association with the plants play a crucial role in the overall health of the plants [21]. But the understanding of the association of the microbiota with the plants is still in the initial stages which is largely due to the large number of interactions, enormous species diversity and complexity in the structure that exists in the rhizosphere. In addition, various microbial behaviors like cooperation, colonization of the niches, infection of the host, resistance to invaders, and invasion by various parasites and pathogens are also reported [22]. Some very good associations in the microbial communities showed mutualistic or altruistic where the donor or recipient may be positively or negatively affected [23]. The microbial diversity in the rhizosphere is very exhaustive and is not yet completely exploited.

Role of Bacteria

Among the rhizosphere flora, one of the most important groups is the bacteria or the plant growth-promoting rhizobacteria (PGPR). This is the most plentiful group among the various microbes, which exist with algae, actinomycetes, fungi and

CHAPTER 9

Endophytes: Distribution, Molecular Characterization and Biodiversity Evaluation

Raj Kumar Gothwal[1,2,*], **Sampat Nehra**[1], **Pooran Singh Solanki**[1,2], **Alok Kumar Varshney**[1,2] and **P. Ghosh**[1]

[1] *Birla Institute of Scientific Research, Statue Circle, Jaipur, Rajasthan, India*

[2] *Department of BioEngineering, Birla Institute of Technology, Mesra, Ranchi, Jaipur Campus, Jaipur, Rajasthan, India*

Abstract: Endophytes are considered all microorganisms present within plants that can be cultured in suitable media (MEA, PDA). In addition to mutualistic and commensalistic symbionts, endophytes could include latent pathogens, latent saprotrophs, and early stages of colonization by mycorrhizal fungi and rhizobia. Endophytes inhabit the interior of plant tissues, causing no harm to the host and do not develop external structures, excluding in this way modulating bacteria and mycorrhizal fungi.

The intimate relationship between endophytic microorganisms and their hosts involves co-evolutionary processes and may influence the physiology of the plant and also interfere with the presence of other endophytes. This endophyte-plant interaction may have been naturally selected during long climatic changes thus allowing a great genetic variability in endophyte populations that open perspectives for the discovery of improved or new enzymes, drugs, and other products with new and useful properties. In this chapter, endophytes, their ubiquitous occurrence, transmission, techniques of isolation, molecular characterization, biodiversity evaluation and future directions for endophytic exploitation have been focused. In the literature, examples have been summarized that show the functional significance and importance of endophytic fungi and bacteria.

Recent studies have demonstrated that these endophytes can be used as vectors to provide new characteristics with biotechnological interest to the host plant. In this aspect, endophytic fungi can be genetically modified and express heterologous genes. They can be used to control pathogens, promote plant growth and produce vitamins, amino acids and vaccines inside the host plant. Therefore, it is extremely important to look at endophytes as microorganisms with biotechnological potential besides their biological role.

Keywords: Endophytes, Heterologous genes, Saprotrophs.

[*] **Corresponding author Raj Kumar Gothwal:** Birla Institute of Scientific Research, Statue Circle, Jaipur, Rajasthan, India; E-mail: rkgothwalbit@gmail.com

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INTRODUCTION

We know that plants are living organisms. However, all botanists, phytopathologists, microbiologists and biotechnologists know that each plant is a natural or agricultural complex community and is colonized by a diversity of microbes both on its outer surfaces (ectophytes) and inner surfaces (endophytes). Therefore, endophytes are those microorganisms that colonize inside plant tissues and part of the microbial community of every plant remains within the tissue of the plant, except that fruiting structures may come up through the surface of the plant tissue. They may be fungi or bacteria that cause asymptomatic infections within plants. This definition excludes pathogenic infections and mycorrhizal fungi. A variety of endophytes may colonize the leaves within a few weeks of leaf emergence and some perennial plants may survive a very long life. The taxonomic distribution and community of endophytes are governed by both temperature and precipitation [1].

In 1886, the German botanist, Anton de Bary, who is considered the father of plant pathology coined the term endophyte. He defined endophytes as "the microorganisms that colonize internal tissues of stem and leaves are endophytes". Later, this definition was revised to specify that infections caused by endophytes are asymptomatic, under these infections, shoots as well as roots may be colonized, and that an endophyte may not persist throughout its life cycle [2, 3]. Endophytes were then defined as asymptomatic microorganisms living inside plants.

Petrini [4] has defined endophytes as microorganisms that inhabit at least for one period of their life cycle, plant internal tissues without causing any apparent damage or harm to the host. Wilson [2] gave an additional definition considering endophytes, as bacteria and fungi that for part of their life cycle or all life, invade/encroach the tissues of living plants and cause asymptomatic and unapparent infections to the plant host. Hallmann and coworkers [5] defined endophytes as "those organisms that may be isolated from surface-sterilized plant parts or extracted from inner tissues and caused no harm to the host plants".

Molecular techniques have shown that microorganisms, which cannot be cultured on regular media and conditions, are found *in vivo* in plants. In the present article, a modified version of the definition [6] will be used: endophytes will be considered as all microorganisms present within plants and these are cultured in suitable media (MEA, PDA, *etc*.) that remain in the inner plant tissues, causing no damage to the host and do not develop external structures, excluding in this way modulating microbes as bacteria and mycorrhizal fungi. This broad definition implies that besides mutualistic and commensalitic symbionts, latent pathogens, and latent saprotrophs and endophytes could include early stages of colonization by mycorrhizal fungi and rhizobia (Fig. **1**). However, functional mycorrhizal fungi are ambiguously excluded, since they are differentiated for nutrient transfer from sources outside of the root [7], and are phylogenetically different from other most of the groups of endophytes [8, 9].

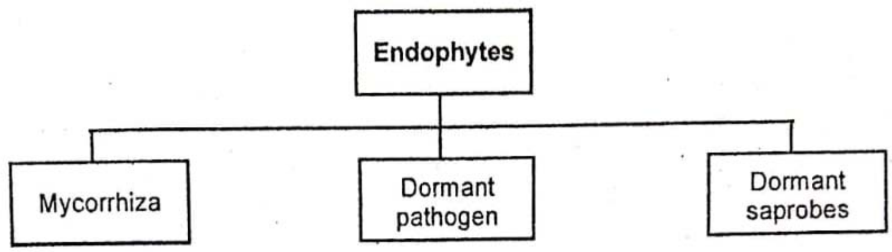

Fig. (1). As part of a fungal community-the mycobiome.

According to Lakshman and coworkers [10], the term endophyte is defined by location and does not address the nature of the relationship with the plant, with the exception of mycorrhiza, which specifies a functional relationship. In 1975, the potential significance of endophytic fungi has become clear, when Charles Bacon discovered that endophytes of pasture grasses in the Clavicipitaceae family were toxic to cattle [11]. Subsequent research work and further findings manifested that both the endophytes and the related toxicity syndromes were widespread, with an estimated approximate cost of $600 million a year to the livestock industry [12].

In general, there are systemic endophytes, in particular, the family Clavicipitaceae is one of the most captivating and economically important examples of plant-fungal interactions, and they have been extensively studied from many perspectives of research. Though they have encouraged a lot of the research on endophytes, they raise expectations of mutualism and functional significance, and that coevolution is often unjustified when applied to other groups of endophytes [13 - 15]. Most of the studies focused on only one of these groups, and some groups consider interactions among them, or between fungi and bacteria. In this view, we have summarized examples that show the functional significance and importance of endophytic fungi and bacteria. These were at the beginning studied as neutral, not causing benefits nor showing detrimental effects to their hosts.

Further, studies indicate that, in plentiful cases, they have a significant role in the protection of host plants, acting against pathogens and predators. From 1981 to 1985, which may be considered a historical period, it was shown that the protection of plants against herbivores can be provided by endophytic

CHAPTER 10

Therapeutic Potential of Endophytes of Medicinal Plants with reference to the Family Zygophyllaceae: A Review

Varsha Sharma[1,*], **Sapana Khandelwal**[2] and **Poonam Meena**[1]

[1] *Department of Botany, University of Rajasthan, Jaipur, Raj., India*

[2] *S.S. Jain Subodh PG Mahila Mahavidyalaya, Jaipur, Raj., India*

Abstract: Medicinal plants have been known for their therapeutic properties for centuries, as they are rich in uncountable and valuable phytochemical compounds. Synthesis as well as different biological aspects of such compounds in plants increases many folds in association with endophytes. These microbial endophytes reside in host plants without causing any symptoms of infection so they are gaining the attention of researchers for research in many fields such as in agriculture, medicines, environmental studies, *etc*. In the recent development of research, the medicinally important family Zygophyllaceae has a limited extent of microbial endophytes' specificity. Very limited studies have been conducted on bioactive compounds isolated from endophytes and their role in health-related benefits. Therefore, this article presents a review of the evaluation of endophytes studied in plants of Zygophyllaceae for the study of bioactive compounds along with various biological activities. This review article will prominently contribute to the exploration of endophytes from medicinal plants of Zygophyllaceae as a source of bioactive and chemically novel compounds.

Keywords: Agriculture, Bioactive compounds, Environmental studies, Microbial endophytes, Zygophyllaceae.

INTRODUCTION

The term endophytes is derived from the Greek word endon-within and phyton-plant, as microorganisms live in the tissues of host plants. Thus, endophytes are such microorganisms that reside in the interior of plant tissues without any symptoms or harm, at least for one period of their life cycle. Endophytes belong to a diverse group of fungi, bacteria or actinomycetes [1, 2]. They have been found to be present in all plant species, residing in their vegetative and reproductive parts. They are capable of entering and flourishing in the plant

* **Corresponding author Varsha Sharma:** Department of Botany, University of Rajasthan, Jaipur India;
E-mail: sharmavarsha1980@gmail.com

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tissues and have many types of interactions within the host plant [3 - 6]. Many times, the association between the plant and its endophytes is mutualistic [7]. Endophytes make their transmission within the host plant vertically as well as horizontally, through seeds or other vegetative propagules, sexual or asexual spores [6]. Endophytes are positioned in the host tissue just below the epidermal layer without any apparent damage to the host.

Microbial endophytes have a potent ability to produce secondary metabolites, which are helpful to guard the host plants against various pathogens, such as insects, pests and other microbes. So endophytes are thought to be advantageous and a possible cause of production or enhancement of novel bioactive secondary metabolites for medicinal and agricultural purposes [1]. They act as a storehouse of various commanding phytochemical compounds such as quinones, alkaloids, amines, phenolic compound sand flavonoids, steroids, saponins, tannins, terpenoids, *etc*. These secondary metabolites are associated with many biological activities such as antimicrobial, anticancer, insecticidal properties, *etc*. Hence, endophytic microbes are an important and rich source of phytomedicines [8]. Nowadays, extensive research is conducted on the isolation and identification of endophytes in any microbial or plant growth medium. Many pharmaceutical products were obtained from these microbes like antibiotics and immunosuppressive compounds. Synthesis of these compounds is fruitful for the normal growth of microbes and is beneficial for host plants [9].

Endophytes are mainly isolated from medicinal plants [10]. Their mutual relationship with their host plant can affect the synthesis of secondary metabolites in such plants. They influence many vital activities of their host plants such as they have an impact on the quality, quantity, and production of medicinally important compounds, helping in promoting their growth, increasing their fitness against the prevailing environment, acting as remediators to abiotic and biotic stresses and also increasing the accumulation of bioactive compounds [11]. Chemical compounds, which were isolated from medicinal plants have diverse and unique structural groups and they have many therapeutic values [3]. Endophytes enhance the resistance of plants to insects and pests. They produce plant hormones and other bioactive compounds including many enzymes and pharmaceutical drugs [8]. These endophytes are also used to synthesize silver nanoparticles and metal nanoparticles that are important in the field of medicine and agriculture [12]. Microbial endophytes also play an important role in oxidative stress protection, heavy metal tolerance, biotic and abiotic stress, and many more. The beneficial relationships of endophytes with plants exhibited in various aspects such as enhanced growth of plants [13, 14], production of various bioactive compounds which provide increased resistance to biotic and abiotic stress to the host plant [15, 16], enhanced production and accumulation of plant secondary metabolites that serve as a potential source of drugs along with various applications in food, agriculture, cosmetics and pharmaceutical industries [17 - 21].

Antibiotics secreted by endophytes are important to prevent the colonization of pathogens, insects and nematodes from infecting plants. The host plants are also benefited from their endophytes having the ability to degrade xenobiotics, natural resistance to soil contaminants, or their action as vectors to introduce a degradative trait in plants, which are helpful in phytoremediation [22].

The importance of these endophytes is also being increased nowadays. Due to the interaction with other microorganisms, they synthesize different kinds of metabolites, thus increasing crop productivity [23]. Bacteria as endophytes provide benefits to plants as they have direct and indirect impacts on the enhancement of plant growth. They affect plant growth directly by the accumulation of essential nutrients such as nitrogen, phosphorus, iron, *etc*., or they synthesize modulated levels of growth-regulating hormones *viz*., auxins, cytokinins, or gibberellins. Bacterial endophytes are also known to produce an enzyme *i.e.* 1-aminocyclopropane-1-carboxylate (ACC) deaminase. This enzyme cleaves the ACC, which is a precursor compound of ethylene. Thus, these bacterial endophytes lower the levels of ethylene in their host plant. They also help in the defence of host plants by the production of antagonistic substances against bacterial or fungal pathogens thus, they promote the growth of the plant indirectly [24].

Mycoendophytes are the most creative group of secondary metabolite producers among endophytes [25]. These mycobionts are isolated from many medicinal plants and are found as a reservoir of novel bioactive compounds. These compounds have a promising role in pharmacology, agriculture and various industries. Endophytic fungi produce novel antimicrobial agents, anticancer compounds, and enzymes. These enzymes can be utilized in different industries for many purposes [24]. Endophytic fungi can produce gibberellins that are essential in improving crop growth and minimizing the harmful impacts of abiotic stresses. Some fungal endophytes have produced many auxins such as indole acetic acid, which enhances root, axillary bud, and flower development and influences other processes [26].

The Family Zygophyllaceae consists of 22 genera and 285 species. Plants of this family have different habits including succulents, herbs, shrubs, shrubs and rarely trees, largely xerophytes or halophytes [27 - 29]. In the family Zygophyllaceae, various plants *viz.*, *Fagonia indica*, *Peganum harmala*, *Tribulus Terrestris*, *Zygophyllum* spp., *etc*., have been studied for the occurrence of endophytic

CHAPTER 11

Antibiotics for Bacterial Disease Management in Plants

M. Hajong[1] and **G. Rajesha**[2,*]

[1] *Soil & Water Conservation Division, Simsanggre, Williamnagar, East Garo Hills, Meghalaya-794111, India*

[2] *ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad-500 030, Telangana, India*

Abstract: Antibiotics are low molecular microbial metabolites that have been used since the 1950s to control bacterial diseases of high-value horticulture and ornamental plants. Bactericidal and bacteriostatic antibiotics were used in agriculture. Although antibiotics were produced primarily for the medical profession and their use was limited by cost, some experiments were conducted soon after they were first produced commercially to determine their effectiveness in the control of plant diseases. In present days, streptomycin and oxytetracycline antibiotics are the most commonly used bacterial disease management in plants. The effectiveness of antibiotics is influenced by a number of factors including antibiotic concentration, method of application, temperature and humidity in addition to host and pathogen factors. The prolonged application of antibiotics in an inappropriate manner is triggering the problem of antibiotic resistance depending on the modes of action, structures, and functional and biochemical properties of antibiotics. A variety of antibiotic resistance mechanisms were expressed in various genes present in pathogens which encode some specific types of enzymes to alter antibiotics into being non-toxic. The main mechanisms of antibiotic resistance were expressed in targeted pathogens by various means of mutation, modification, and replacement of various genes and target sites of antibiotics. The rational use of antibiotics is one of the key approaches to increasing the efficacy of antibiotics and prevention of resistance in future for the bacterial disease management.

Keywords: Antimicrobial metabolites, Antibiotic resistance, Actinomycetes, Bactericidal antibiotics, Bacterial diseases, *Streptomyces*.

INTRODUCTION

Plant diseases caused by bacterial pathogens are a serious hindrance to crop production that induced substantial annual crop losses worldwide [1]. A variety of

* **Corresponding author G. Rajesha:** ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad-500 030, Telangana, India; E-mail: rajesha@millets.res.in

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integrated disease control measures were used to reduce crop losses. Among the integrated disease management practices, the use of antibiotics is one of the effective measures for the management of bacterial diseases. Soon after the introduction of antibiotics in 1950, in human medicine, the power of these wonder drugs in controlling plant diseases was also explored [2 - 5]. Antibiotics can be defined as the secondary metabolites produced by certain microorganisms as well as chemically synthesized or semi-synthesized analogous compounds that could inhibit the growth and survival of other target microorganisms [6]. The application of antibiotics in plant disease management is not new as they have been very well used on crops like apples and pears against some bacterial diseases, whereas the study on the extent of adoption of antibiotics is very limited and is largely unknown due to many factors [7].

Antibiotics are the best option for reducing the pathogen's population size and limiting disease outbreaks in the disease management system when there is a lack of resistant crop varieties. Therefore, the use of antibiotics for the control of plant diseases has received increasing attention [8 - 10]. For the effectiveness of antibiotics against plant diseases, they should be active on or inside the plant, tolerate oxidation, rainfall, high temperature, and UV irradiation, and be safe to plants and for resistant pathogens only at a low or non-detectable rate.

However, the use of antibiotics in plant disease management strategy was toughened by various factors such as the lack of efficacy at lower concentrations, phytotoxic at higher concentrations and higher expenses as compared to other prevailing methods of disease management strategy. Thus, even though antibiotics *viz*., penicillin, streptomycin, chloramphenicol, oxytetracycline, and aureomycin were tested for plant disease management in the late 1940s [10, 11], only streptomycin and oxytetracycline were eventually deployed in bacterial disease management in plants. The present chapter will discuss the major antibiotics with respect to their potential use against bacterial disease management and their mode of action upon their penetration into the plant.

CLASSIFICATION AND TYPES OF ANTIBIOTICS

The classical definition of antibiotics given by Waksman, who discovered streptomycin in 1944, is "a compound produced by a microbe with killing or growth-inhibiting activity against other microbes" [12]. Enormous numbers of known antibiotics were produced by actinomycetes (8700), bacteria (2900), and fungi (4900) [13]. Only less than 1% of microscopically counted bacteria could be cultured on usual culture media [14]. Many bacterial species have been found for antibiotics' production but almost one-third of the bacterial divisions have no cultured representatives for antibiotic production and were known only through rRNA sequences [15]. Therefore, it can be presumed that the majority of antibiotics produced *in vitro* in the environment are still unexploited to the full extent [16]. Microbial genome analysis disclosed that an enormous number of hidden antibiotic gene clusters encoding are still unknown antibiotics. The production of secondary metabolites with broad-spectrum activity has been reported in many bacterial biocontrol agents belonging to *Agrobacterium*, *Bacillus*, *Pantoea*, *Pseudomonas*, *Serratia*, *Stenotrophomonas*, *Streptomyces*, and many other genera. Several antibiotics were produced only when a microbial population attains sufficient thresholds level. This quorum-sensing phenomenon was well explained for phenazine-producing *Pseudomonas*. Genomic data also explain that *Pseudomonas* have the ability to generate several still unknown secondary metabolites with potential antimicrobial activity.

Antibiotics are classified into two major classes based on the type of action of antibiotics.

1. Bactericidal antibiotics: These antibiotics kill the bacterial population by inhibiting cell wall synthesis, *e.g.*, β-lactam antibiotics such as penicillin, cephalosporins, and vancomycin (Table **1**).

Table 1. Mode of action of antibiotics.

Antibiotics Class	Antibiotics	Mode of Action and Mechanism
Cell wall synthesis		
Bactericidal: β-lactams Lipopeptides	Penicillin and cephalosporin Polymycin, vancomycin, and bacitracin	Block cross-linking *via* competitive inhibition of the transpeptidase enzyme [17, 18].
Protein synthesis inhibitors		
Bactericidal: Aminoglycosides	Gentamicin and streptomycin	Irreversible binding to 30S subunit: streptomycin inhibits protein synthesis and binds within the ribosome to four nucleotides of the 16S RNA and the ribosomal protein S12 [17 - 21].
Bacteriostatic: Tetracyclines	Oxytetracycline and tetracycline	Act upon the conserved sequences of the 16S rRNA of the 30S ribosomal subunit to prevent binding of tRNA to the A site [17, 18, 22 - 24].
Bacteriostatic: Macrolides Amphenicols	Erythromycin and Chloramphenicol	Interacts with the conserved sequences of the peptidyltransferase cavity of the 23S rRNA of the 50S subunit. Hence, it inhibits the protein synthesis by preventing the binding of tRNA to the A site of the ribosome [17, 18, 24, 25].

CHAPTER 12

Microbial Proteases: Importance in Crop Yield Improvement

Prachi Awadhiya[1,*], **Prachi Singh Baghel**[1] and **Neeraj Verma**[1]

[1] *Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, 485001 (MP), India*

Abstract: Proteases are degradative enzymes, which catalyze the total hydrolysis of proteins. Advances in analytical techniques have demonstrated that proteases conduct highly specific and selective modifications of proteins such as the activation of zymogenic forms of enzymes by limited proteolysis blood clotting and processing and transport of secretory proteins across the membranes. The main sources of proteases are animals, plants, and microbes. Proteases from microbial sources are preferred to enzymes from plant and animal sources since they possess almost all the characteristics desired for their biotechnological applications. Proteases are further categorized as serine proteases, Aspartic proteases, cysteine proteases or metalloproteases – depending on their catalytic mechanisms. Moreover, proteases are also classified based on their pH –being acidic, neutral or alkaline proteases. Microbial proteases have numerous applications in different sectors like leather, detergent, food, photographic industry, *etc*.

Keywords: Classification, Industrial uses, Proteases, Sources.

INTRODUCTION

Proteases are a universal entity that is found everywhere, namely, in plants, animals, and microbes. The peptide bond present in the polypeptide chain of amino acids is hydrolyzed by means of proteases [1]. Proteases are degradative enzymes and show specificity and selectivity in protein modification [2]. Proteases are an integral component of existing life on earth, such as animals, plants, and microbes. By a process of fermentation, proteases can be isolated and purified in a relatively shorter period of time, exhibiting high substrate specificity and catalytic activity [3, 4]. It is estimated that proteases account for 1–5% of the genome of infectious organisms and 2% of the human genome [5]. According to researchers, proteases control the activation, synthesis, and turnover of proteins to regulate physiological processes [6]. Different physiological processes, such as

* **Corresponding author Prachi Awadhiya:** Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, 485001 (MP), India; E-mail: neerajverma.2008@yahoo.com

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formation, birth, aging, and even death are regulated by proteases [7, 8]. Proteases are one of the most important groups of industrial enzymes that have been studied extensively in recent years [9]. Proteases [EC 3.4] are a group enzymes that occur in all life forms, and execute a large variety of complex physiological and metabolic functions in living systems like cell division, signal transduction, digestion, blood pressure regulation, apoptosis and several others [10]. The proteases intended for biotechnological processes must be robust enough and have the capability of kinetic and structural adaptations under extreme industrial microenvironments, *e.g.*, extremes of temperatures, pH, and presence of inhibitors [11].

SOURCES OF PROTEASE

Since proteases are physiologically necessary for living organisms, they are ubiquitous, being found in a wide diversity of sources such as plants, animals, and microorganisms.

Plant Proteases

The use of plants as a source of proteases is governed by several factors such as the availability of land for cultivation and the suitability of climatic conditions for growth. Moreover, the production of proteases from plants is a time-consuming process. Papain, bromelain, keratinases, and ficin represent some of the well-known proteases of plant origin.

Animal Proteases

The most familiar proteases of animal origin are pancreatic trypsin, chymotrypsin, pepsin, and rennins [12, 13]. These are prepared in the pure form in bulk quantities. However, their production depends on the availability of livestock for slaughter, which in turn is governed by political and agricultural policies.

Microbial Proteases

Although protease-producing microorganisms, plants, and animals have a cosmopolitan distribution in nature; the microbial community is preferred over others for the large scale production of proteases due to their fast growth and simplicity of life for the generation of new recombinant enzymes with desired properties. Microorganisms account for a two-third share of commercial protease production in the enzyme market across the world [14]. Proteases play a decisive role in detergent, pharmaceutical, leather, food, and agricultural industries. Currently, the estimated value of the global sales of industrial enzymes is over 3 billion USD [15] of which proteases account for about 60% of the total sales [2, 16]. Proteins are degraded by microorganisms, and they utilize the degradation products as nutrients for their subsistence. Degradation is initiated by proteinases (endopeptidases) secreted by microorganisms followed by further hydrolysis by peptidases (exopeptidases) at the extra- or intra-cellular locations. A number of proteases are produced by microorganisms depending on the species of the producer or the strains, even belonging to the same species. Neutral and alkaline proteases hold a great potential for application in the detergent and leather tanning industries due to the increasing trend in developing environment-friendly technologies [2]. Alkaline proteases have numerous applications in the food industries, silver recovery from X-ray films and several bioremediation processes.

Fungal Proteases

Fungal proteases magnetized the interest of researchers due to their high diversity, broad substrate specificity, and stability under extreme conditions; it offers an advantage of the separation of mycelium by simple filtration. Fungal proteases can conveniently be produced in the solid-state fermentation process and can also be used for modifying food proteins. The different alkaline proteases producing fungal species are *Aspergillus candidus* [17] and *A. flavus* [18].

Bacterial Proteases

Bacterial alkaline proteases have more commercial importance in laundry, food, leather and silk industries due to their high production capacity and catalytic activity. Bacterial alkaline proteases are characterized by their high activity at alkaline pH [8 - 12], with optimal temperatures between 50˚C and 70˚C. These properties of bacterial alkaline proteases make them suitable for use in the detergent industry. Prominent bacteria producing proteases are *Alteromonas* sp [19]. and *Lactobacillus helveticus* [20].

Viral Proteases

Viral proteases have gained importance due to their functional involvement in the processing of proteins of viruses that cause certain fatal diseases such as AIDS and cancer. Serine, aspartic, and cysteine peptidases are found in various viruses [21]. All of the virus-encoded peptidases are endopeptidases; there are no metallopeptidases. Retroviral aspartyl proteases that are required for viral assembly and replication are homodimers and are expressed as a part of the polyprotein precursor. The mature protease is released by autolysis of the precursor. Extensive literature is available on the expression, purification, and enzymatic analysis of retroviral aspartic protease and its mutants [22].

CHAPTER 13

Biocontrol Products to Control Plant and Animal Diseases

Sirisha Thakare[1], **Pravin Khaire**[1,*], **Mukund Dawale**[1], **Someshree Mane**[1] and **Sanjay Kolase**[1]

[1] *Department of Plant Pathology and Agricultural Microbiology, PGI, MPKV, Rahuri (M.H.)-413722, India*

Abstract: Plant and animal diseases continue to pose a significant and long-term shortage of food, food hygiene, foreign business, bio-diversity, and the ecological system. Numerous questions, such as global warming, regulatory developments, changes in the geographical size and concentration of farm animals' assets, and increased trade, create this an excellent time to assess the level of research on disease effect and biocontrol product management and control. This paper examines the rationale for conducting an integrative study to investigate the management practices of contagious plant and animal diseases. Finally, the organisation of the content under this chapter provides a picture of current plant and animal diseases.

Keywords: Biocontrol products, Bacteriophage, Phyto and animal diseases, Remediation, Rnai, Secondary metabolites.

INTRODUCTION

Plant or animal diseases outbreaks are not purely natural phenomena. Human actions play a significant role in the dissemination and outbreak of diseases. Diseases, in contrast, have a large impact on humans, and great effort is expended in disease management. As a result, it is hard to differentiate between the natural events of disease and the social phenomena of disease sources, impacts, and regulation. Nonetheless, our knowledge of animal and plant diseases is riven by a significant gap between the scientific and social sciences—a gap that is ingrained in variations in research methodologies, strategies, and perspectives. The aspects of reality fragmentation impede the progress in understanding and treating diseases. The goal of this chapter is to get united researchers from many academic fields to provide new insights into current animal and plant disease risks. In this introductory work, we explain the intricate relationships between the natural and

* **Corresponding author Pravin Khaire:** Department of Plant Pathology and Agricultural Microbiology, PGI, MPKV, Rahuri (M.H.)-413722, India; E-mail: pravinkhaire26893@gmail.com.

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social phenomena in animal and plant diseases and make the case for a multidisciplinary approach to disease prevention that combines natural and social sciences.

Biological control (BC) is a way of limiting the effects of hazardous animals, pathogens, and plants by employing other beneficial species that inhibit harmful organisms, such as microbes, insects, and plants. Predation, parasitism, pathogenicity, and competition are all examples of basic ecological interactions between species that the approach takes advantage of. BC is now predominantly employed in agriculture to combat pests. The merits of biological control include the elimination of artificial substances and the rarity of pathogens or animals that develop resistance to biological control agents (BCAs). Biological pest control (BPC) is a crucial part of integrated pest management. The development of resistance to most of the existing synthetic pesticides by the leading pests has sparked interest in biological techniques for livestock and poultry pest management. Organic methods, as well as those that boost animal welfare by reducing animal numbers and enabling greater freedom of movement, have seen a significant increase. New tools and tactics to manage pest problems are essential in such systems, particularly organic ones.

Biological control is described as “a certain action of one life form that minimizes the detrimental impact on the other” [1]. Another definition of biocontrol is “the analysis of parasites, predators, as well as diseases for the regulatory oversight of host densities” [2]. Natural control differs from BC in that it does not necessitate human intervention. The term “EPF” refers to fungi that infect and kill arthropod pests (insects, ticks, and mites). There are approximately 750 EPF species, with the majority belonging to the phylum Ascomycota and a few belonging to the phylum Zygomycota and Ascomycotina [3]. EPFs from the genera *Metarhizium* and *Beauveria* are the most commonly studied, and they're increasingly being used in commercial arthropod repellents. *Metarhizium anisopliae* and *Beauveria bassiana* are also mosquito repellents [4]. They infect mosquitos early in their lives and kill them between 3 to 14 days later, depending on the exposure dose and fungal isolate [5]. Commercially available bioproducts based on *B. bassiana* include Mycotrol O (Emerald BioAgriculture), Naturalis Home and Garden (H&G), Naturalis L (Troy BioSciences, Inc.), and Biosect (Kafr El Zaya-KZ Chemicals, Egypt). Biosect was used to control mosquito larvae of *Musa domestica* and *Culex pipiens in vitro*, and total larval mortality was nearly 100% [6]. Insect control is also done with fungi such *Leptolegnia* spp., *Coelomomyces* spp., *Hirsutella thompsonii*, *Nomuraea rileyi*, and *Verticillium lecanii* [7]. *Beauveria bassiana* and *Metarhizium anisopliae*, the two fungi, have attracted the most attention and have been tested against most of the important cattle and poultry pests.

WHY IS BIOCONTROL (BC) COMMERCIALIZATION NEEDED?

The advert use and implementation of biological disease control have been slow, owing primarily to their variable success in the field under a wide range of environmental conditions [8 - 11]. Numerous biocontrol agents work well in the laboratory and greenhouse but not in the field. This issue can only be fixed by increasing the knowledge of the external factors that influence biocontrol agents [8 - 11]. In regard to this issue, there has been little expenditure in the production and development of commercial products of biocontrol-active microorganisms, owing to the high costs of developing, checking, registering, and advertising these products [12, 13].

Biological control agents (BCAs) are commonly formulated as wettable powders, dusts, granules, and aqueous or oil-based liquid products containing various organic and mineral carriers [13] (Table **1**). A variety of biologically based products are commercially available for the management of harmful microorganisms that cause plant diseases [13] (Table **2**). A large number of companies are also introducing new products that are currently being registered. Several of these businesses are small, privately held enterprises with a scarce line of products. Some are traded publicly and have large market capitalizations. Furthermore, bigger companies with more diversified product lines which include a wide range of agrochemicals and biotechnological products have performed an essential role in the advancement and advertising of plant pathogen control products [13].

Table 1. List of base materials/carriers used for mass production of fungal biocontrol agents [14].

Biocontrol Agent	Base Material(s)	Form of Formulation
Trichoderma harzianum	Black gram shell, shelled maize cob, coir-pith, peat, gypsum, coffee fruit skin + biogas slurry, coffee husk, coffee-cherry husk, fruit skin and berry mucilage, molasses-yeast, molasses-soy, molasses-$NaNO_3$, molasses-KNO_3, mushroom-grown waste, poultry manure, soil, sorghum grain, spent tea leaf waste, sugarcane straw, wheat bran + biogas manure (1:1), and wheat-bran + kaolin	Powder and pellets
T. viride	Barley grains, black gram shell, shelled maize cob, coir-pith, peat, gypsum, coffee husk, fruit skin and berry mucilage, mushroom grown waste, mustard oil cake, neem cake + cow dung, poultry manure, spent tea leaf waste, sugarcane straw, talc, and vermiculite + wheat bran + HCl	Pellets

CHAPTER 14

Microbiological Management of Composting Processes

G.N. Chattopadhyay[1,*] and **Gourab Roy**[2]

[1] *Seacom Skills University, Kendradangal-731236, Birbhum, West Bengal, India*

[2] *Department of Zoology, M.B.B. College, Agartala-799004, Tripura, India*

Abstract: Lack of sustainability in agricultural production owing to the gradual deterioration in soil health is emerging as a major concern in Indian agriculture. This behavior has largely been attributed to over-dependence on mineral fertilizers and limited use of organic matter during the last several decades. To come out from this situation, the necessity of larger incorporation of organic materials in the agricultural soils is being emphasized at different levels. Since the availability of traditional organic manures is gradually reducing in the country, while a plentiful amount of wide ranges of biodegradable organic waste materials are being generated every day, growing attention is now being paid to the recycling of these wastes as organic manures for improving the health conditions of our arable soils. However, most of these organic wastes cannot be directly added to the soils due to some limitations in their chemical as well as biological properties and, therefore, adoption of various composting processes is being suggested for this purpose. With the present thrust and encouragement from the Government on waste recycling under the "*Swachh Bharat Yojna*", a good number of small and medium-scale industries have come up in this composting sector and many more are in the pipe line. Now, composting is basically a process of microbiological degradation of various organic materials to form humified end products along with the release of various nutrient elements. Hence, for successful implementation of any waste management program through composting, a thorough knowledge of the roles of various microorganisms in the decomposition of varying natures of organic wastes, their behaviors, successions, relative efficiency levels, *etc.*, need to be understood thoroughly. In this article, various aspects of composting microbiology have been discussed with special reference to the occurrence and behavior of different microbes during the process of composting. Several aspects like the relative efficiency of the microorganisms in degrading varying components of organic wastes, microbial acceleration of composting, biological fortification of compost quality, *etc.*, have been discussed to provide a gross idea for efficient microbiological management of the composting process.

* **Corresponding author G.N. Chattopadhyay:** Seacom Skills University, Kendradangal-731236, Birbhum, West Bengal, India; E-mail: gunin.c2010@gmail.com

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Keywords: Acceleration, Composting, Efficiency, Fortification, Microbial processes, Successions.

INTRODUCTION

India has witnessed significant success in the field of agricultural production since its independence. While the food grain production of the country was merely 55 M tons in 1950-51, just after the independence, the said production during 2018-19 has reached a value of 285 M tons [1] recording a more than five times increase. Since the growth and production of any plant require adequate amount of different plant nutrients in available forms, such an escalation in food production along with other agricultural products could only be achieved when large quantities of different plant nutrients were made available to the cultivated crops. Fertilizers being an easy source of various plant nutrients in available forms, mineral fertilizers have played an important role in the progress of Indian agriculture resulting in manifold increase in its use (Fig. **1**). However, in spite of such increasing uses of fertilizer inputs, a trend of gradual stagnation in the per capita availability of food grains in the country has been observed in recent years. Although a consistent increase in the population is a major reason behind such a scenario, yet declining use efficiency of mineral fertilizers is also being attributed as another important factor for such behavior.

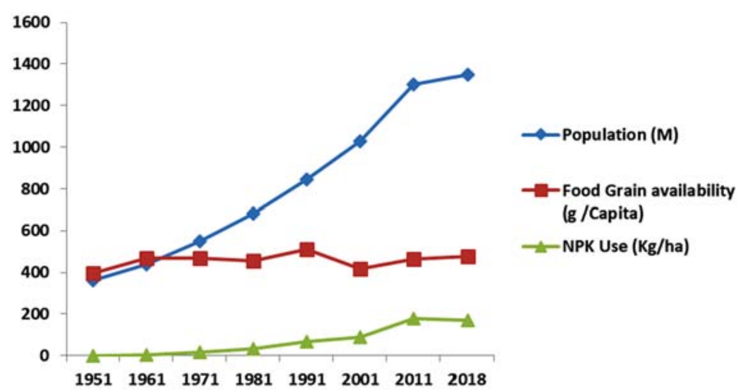

Fig. (1). Post independence scenario of Indian agriculture.

Declining soil health is often cited as one of the reasons for such unsustainability in crop yields. The imbalanced use of nutrients, coupled with their low efficiency (Table **1**) and neglecting the use of organic manures, has often led to multi-nutrient deficiencies in many areas over time. As a consequence, increasing uses of fertilizer inputs are now being required to achieve the required quantum of food production. These practices are behaving as a vicious cycle leading to furthering the problem of deterioration in soil health. As a result, a considerable decline in soil and crop productivity is being reported from different parts of the country, particularly from the intensively cultivated areas [3]. Under this context, increased attention is now being paid to improve soil health with the objective of sustaining the productivity of arable soils.

Table 1. Nutrient use efficiency in Indian agriculture [4].

Nutrient	Use Efficiency (%)
Nitrogen	30-50
Phosphorus	15-25
Potassium	60-70
Sulphur	8-12
Micronutrients	1-4

Soil Health

Soil health is commonly defined as the continued capacity to function as a vital living system, with the ecosystem and land use boundaries, to sustain biological productivity, maintain or enhance the quality of air and water, and promote plant, animal and human health [5]. For various practical purposes, this concept of soil health may be considered as an elaboration of soil productivity [6], commonly explained as "the capacity of a soil to produce crops" [7]. Number of physical, chemical and biological properties which can perform as indicators of soil health were identified and discussed by many researchers [6, 8, 9] in India. While discussing the roles of various soil quality attributes, many of these workers suggested the importance of using a minimum data set for the assessment of soil quality. We have used their observations to rely on some easily accessible parameters, which should grossly indicate the health status of most of the soils (Table **2**). Any shortcoming with regard to these attributes is likely to exert severe adverse effects on the productivity of the arable soils.

Table 2. Some commonly used indicators of soil health [6, 8].

Physical	Chemical	Biological
Soil Structure	Availability of plant nutrients	Microbial biomass carbon
Infiltration	pH	Respiration
Bulk density	Electrical conductivity	Soil enzymes
Soil crusts	Cation exchange capacity	Earthworms
Water holding capacity	Organic carbon content	Easily mineralizable nitrogen

CHAPTER 15

Microbial Origin Nematicides: An Eco-friendly and Potent Tool to Management of the Plant-Parasitic Nematodes

Rashid Pervez[1,*], **Mohammad Danish**[2] and **Neeraj Verma**[3]

[1] *Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi-11012, India*

[2] *Department of Botany, A.M.U., Aligarh-202001 (Uttar Pradesh), India*

[3] *Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, 485001 (MP), India*

Abstract: Plant-parasitic nematodes (PPNs) are a serious threat to the quantity and quality of many economic crops around the world. As a result of rising dissatisfaction with the hazards of chemical nematicides, interest in microbial control of PPNs is developing, and biological nematicides are becoming an important component of ecologically acceptable management strategies. Bionematicides can be employed in integrated nematode management (INM) programs to maximize their benefits, with techniques that make them complementary or superior to chemical nematode control approaches. This is especially relevant in integrated pest control systems because bionematicides can operate synergistically or additively with other crop inputs. bionematicides and other pesticides should be used in a more coordinated manner. This is especially relevant because numerous bionematicides are already or will soon be commercially available. It is still necessary to identify research objectives for using fungal and bacterial nematicides in sustainable agriculture, as well as to get a better knowledge of their ecology, biology, mode of action, and interactions with other agricultural inputs. As a consequence, utilizing a microbial nematicide from the stated category as a plant-parasitic nematode biocontrol agent is a viable long-term biocontrol technique in agriculture.

Keywords: Biocontrol, Management, Nematicide, Plant-parasitic nematodes.

INTRODUCTION

Agriculture production in India has sustained losses due to several reasons. Among them, one of the major constraints is diseases, which are the limiting factor in the cultivation of crops. Among them, the disease caused by plant-parasitic nematodes (PPN) is one of the constraints in reducing both the quality

* **Corresponding author Rashid Pervez:** Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi-1012, India; E-mail: rashidpervez2003@gmail.com

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and yield of the crops. They cause 21.3% crop losses amounting to 102,039.79 million ($ 1,577 million) annually; the losses in nineteen horticultural crops were assessed at 50,224.98 million, while for 11 field crops, it was estimated at 51,814.81 million. Rice-rot nematode *Meloidogyne graminicola* was economically most important causing the yield loss of 23272.32 million in rice. Citrus (9,828.22 million) and banana (9,710.46 million) among fruit crops; and tomato (6,035.2 million), brinjal (3,499.12 million), and okra (2,480.86 million) among the vegetable crops suffered comparatively more losses [1, 2]. It has been estimated that global losses amount to $78 billion due to RKN [3].

Plant-parasitic nematodes are considered to be a hidden enemy of crops due to their microscopic size, hidden habitats, and lack of manifestation of clear-cut symptoms on the aerial parts of the plants. PPN invades the plant's roots, where each nematode establishes a feeding site [4, 5]. This is an intricate developmental process, which leads to major changes in root structure and metabolism *i.e.* the formation of root galls, syncytia, giant cells, lesions, *etc*. Root-knot nematodes (RKN), *Meloidogyne* spp., are the most damaging nematodes of crop production worldwide. The impact of this nematode genus is enhanced by its wide host range of more than 5,000 plant species and it causes severe economic losses in many agricultural and horticultural crops [6]. One or more nematode pests are always associated with every crop, which causes economic loss to crops; their control is one of the major requirements for increasing crop productivity [7, 8].

Pesticides are currently being used to manage these nematode pests leading to environmental and health concerns and resulting in the suppression of other naturally occurring biocontrol agents as well as resistance in their nematodes. Because nematicides are living systems, developing commercial bionematicidal products presents a variety of challenges. Some workers have gone into great detail about problems with their culture and formulation, the variable gap between laboratory and field performance, potentially negative effects on non-target or beneficial organisms, and expectations of broad-spectrum activity and quick efficacy based on experience with synthetic chemical nematicides [9 - 11].

The importance of biological nematode pest management is expanding, as evidenced by multinational businesses' significant investment in research as well as their acquisitions of minor biotechnology companies with microbial product portfolios [12, 13]. Among various bionematicides, bioproducts including antifungal and antibacterial agents rank first [14]. Various areas of bionematicidal production and usage have advanced rapidly during the last two decades. This was notably significant for the development of *Pasteuria* spp. *in vitro* mass culture and new, easy-to-use product formulations. Nonetheless, there is a pressing need to position these bionematicides as more effective and trustworthy PPN fighting agents. This tendency is presently manifesting itself in a variety of ways, such as research into their uses, enhanced shelf-life, mass culture, and interactions with other biotic and abiotic elements, as well as biocontrol integration with other management strategies.

MANAGEMENT OF PLANT NEMATODES THROUGH MICROBIAL ORIGIN NEMATICIDES

Many soils inhibit microorganisms like fungi, bacteria, protozoans, viruses, turbellarians, enchytraeids, mites, predatory nematodes, collembolans, tardigrades, *etc.*, are parasites, predators, or antagonistic to plant-parasitic nematodes. These microorganisms have been exploited as biocontrol agents for the management of PPN infesting several agricultural and horticultural crops. The increasing thrust towards sustainable agriculture and integrated pest management has led to biological control emerging from its status as a fringe sector to being viewed as an intrinsic part of crop protection. A brief description of the most promising microbial-origin nematicides is furnished below.

Paecilomyces lilacinus (*Purpureocillium lilacinum*)

This is an opportunistic fungus that is common in many soils. This fungus parasitizes root-knot nematode eggs and prevents them from hatching. Inoculation of this fungus surrounding the root zone could reduce root loss caused by nematode infection and increase plant growth. The polyphyletic structure of the genus *Paecilomyces*, as well as the evolution of this taxonomic research, are critical for producing agricultural microbial formulations [15]. *Paecilomyces phialides* have a broad base and an extended neck, and hyaline to yellowish septatehyphae with smooth walls and verticillated or irregularly branching conidiophores. *Paecilomyces* is a fungus that can grow in a broad variety of temperatures and substrates and has a high sporulation rate. As a result, its fast replication assures the creation of commercial formulations is practical and economical [16].

Paecilomyces have been extensively explored as a nematophagous fungus and may be found in a range of biological formulations for agricultural usage [17]. *Paecilomyces* spp. have been found to serve as nematicidal agents against *Meloidogyne* spp., as well as other genera such as *Globodera* sp. [18], *Rotylenchulus* sp., *Heterodera* sp., *Xiphinema* sp., and *Pratylenchus* sp. [19]. *Paecilomyces* spp. can infect eggs, immature nematodes, or adult nematodes at various stages of development. The principal barrier against parasitic agents is the plant nematode eggshell, which is resistant to both chemical and biological nematicides. *Paecilomyces* sp. can secrete enzymes that break down this barrier and use nematode parasitism mechanisms to do so [20, 21].

CHAPTER 16

Major Viruses Infecting Temperate Fruit Crops and Their Impact on the Fruit Industry

Sumiah Wani[1], Mohd Ashaq[2], Sumaira-Hamid[3], G.H. Dar[4], Asha-Nabi[1], Mushatq Ahmad[4], B.A. Padder[1] and **Mehraj D. Shah[1,*]**

[1] *Plant Virology and Molecular Pathology Lab., Division of Plant Pathology, FoH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar-190025, Jammu and Kashmir, India*

[2] *Department of Botany, Govt Degree College, Thannamandi, District Rajouri, Jammu and Kashmir, India-185132*

[3] *Department of Biosciences, Integral University, Luchnow-226 026, Uttar Pradesh, India*

[4] *Centre for Advancement of Applied Sciences, Shalimar, Srinagar-190025, Jammu and Kashmir, India*

Abstract: Several plant viruses infecting temperate fruit crops are extremely infectious and have devastating effects on the host trees. They all have a significant effect on yield and yield-related efficiency. Others cause a slew of problems, necessitating a large sum of money to save these infectious diseases from wreaking havoc. Yield and other economic losses are the most visible manifestations of this effect. These viruses cause economic loss to the farmers/producers and consumers by affecting plant growth and reproduction, causing sterility, yield and/or quality reduction, increased susceptibility to other stresses, crop failure, loss of aesthetic value, quarantine, and the need for eradication of the infected plants, thus increasing the cost of control measures as well as detection programs. Since future yield and risks are so unpredictable, losses incurred by any viral disease cannot be calculated explicitly. Experimental evaluation of the losses due to viral diseases is difficult because the infection of safe, controlled plants is rarely possible, and inoculations under vector-proof conditions do not adequately represent what occurs in natural conditions. Viruses are also unusual, and their structures are deceptively simple. However, this simplicity leads to a stronger reliance on the host, and the two have a complicated relationship. This complicates plant-virus management strategies as well as the damage caused by them. Plant virus control systems rely on our knowledge of the virus-vector/host relationship and will remain one of the most difficult tasks faced by plant virologists, growers, and nurserymen in the future.

* **Corresponding author Mehraj D. Shah:** Plant Virology and Molecular Pathology Lab, Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar- 190025, J & K, India; E-mail: mehraj547@rediffmail.com

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Keywords: Economy, Fruit crops, Loss, Soil-borne viruses, Temperate viruses, Viral diseases.

INTRODUCTION

Temperate fruit trees, including pome (apple, quince, and pear) and stone fruits (apricot, peach, plum, almond, and cherry), belong to the family Rosaceae. In addition to these, the Vitaceae family's grapevine (*Vitis* spp.) is a major fruit crop grown in temperate to tropical regions with global socioeconomic importance [1]. These fruits are of vital importance to human nutrition and can reduce the risk of cardio-vascular diseases, including some cancers, when consumed regularly. Almost half of the global temperate tree fruit production is from China, Turkey, the United States, Brazil, Italy, and Spain. China is the world's largest producer of apples, peaches, and plums, while Turkey is the largest producer of cherries and apricots. The spatial spread of temperate horticulture crops is heavily affected by the environment. Specific climatic conditions, like the requirement of a cool temperature for breaking dormancy, are the most important factors that affect their development. Chilling requirements vary depending on species; for example, apples have the highest chilling requirements followed by apricots and peaches. Different cultivars of the same plant can respond differently to different climatic conditions, resulting in greater temperature adaptability. Climate change has a major influence on quantitative and qualitative aspects of the fruit industries. The existence of viral diseases and their carriers or vectors is also affected by the environment, which may enhance the threat of unusual pathogens being introduced and change the distribution environments of various pests. This is also true with widely transmitted systemic infections, including pome and stone fruit viruses. Virus-related losses in pome and stone fruits are normally undetectable and untreated. Some viruses are mostly inactive in some of their hosts, causing slow growth and development in plants, reducing fruit size and the number of fruits, having a modified product structure and shortened life period, or having some other unnoticed results [2]. The horticulture industry has expanded dramatically over the last 50 years, becoming one of the most significant economic divisions of agriculture. The most important pome and stone fruits are: apple (*Malus* x *domestica* Borkh.), pear (*Pyrus* spp.), quince (*Cydonia oblonga*), plum (*Prunus domestica*), peach or nectarine (*Prunus persica*), cherry (*Prunus avium*), apricot (*Prunus armeniaca*), and almond (*Prunus dulcis*). In terms of production and distribution in India and elsewhere, apples are the most important temperate fruit grown worldwide, followed by pears, peaches, apricots, plums, almonds, and cherries. Viruses are transmitted by various vectors, and most of them spread by the vectors like insects, soil-inhabitant fungi, and nematodes, and some viruses are educated from the infected roots of the plants and transmitted in an abiotic manner without any vector. The persistence of such viruses for a long time in the soil results in frequent disease occurrence, and hence their management strategy is really very difficult.

VIRUSES INFECTING TEMPERATE FRUIT CROPS

Temperate fruit production, like other crops, is inhibited by a variety of biotic and abiotic constraints, resulting in poor tree health and low productivity. Fruit crops grown in temperate climates are vulnerable to a variety of fungal and bacterial infections as well as a wide range of insect pests. Mildew, scab, leaf curl, aphids, and other diseases/pests of temperate fruits are widespread in India [3]. Amongst the various factors responsible for low productivity, infection by viruses has also been discovered to be a limiting factor in growing healthy orchards of temperate fruits. Viruses are important but understudied pathogens in fruit crops in different countries, especially in India. Most of the temperate fruits are propagated vegetatively by budding or grafting the desired cultivar (scion-wood) on a suitable rootstock, which may be a seedling-or clonal-rootstock. The viruses that infect these fruits are transmitted primarily by the use of infected bud-/graft-wood during propagation, and once infected, a plant cannot be healed by any chemical therapy. The increased international exchange of propagative materials and their end goods without adequate quarantine procedures has resulted in the accelerated emergence and widespread geographic dissemination of viruses. Soil-borne viruses such as nematodes or fungi-transmitted viruses also spread by the geographical exchange of the plant material under poor quarantine procedures, especially when planting material is contaminated with the infected soil or microbes carrying viruses.

Viruses Infecting Pome Fruits

Pome fruit trees comprised of apple and pear have been found to be infected by twenty-one viruses belonging to twelve genera and nine families. *Apple mosaic virus* (ApMV), *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), and *Apple stem grooving virus* (ASGV) are the most economically important viral diseases infecting pome and stone fruits. The remaining viruses that infect pome fruit trees are of minor importance or there is insufficient research evidence to determine their relevance [4].

Apple Chlorotic Leaf Spot Virus (ACLSV)

The *Apple chlorotic leaf spot virus* (ACLSV) is a member of the *Trichovirus* genus and the Betaflexiviridae family. ACLSV is a filamentous flexuous particle virus with a length of 680–780 nm and a diameter of 12 nm. It has a lot of molecular diversity, and many virus isolates with different pathogenicities have been identified. This virus for the first time was isolated from apple trees after

CHAPTER 17

Nutritional Value and Nutraceutical Properties of Mushrooms

Diwakar Bahukhandi[1,*]

[1] *Division of Mycology and Plant Pathology, ICAR- Indian Agricultural Research Institute, Pusa, New Delhi, India*

Abstract: Mushrooms are fleshy or macro fungi, belong to a special group in biological science, the Mycota and their descriptive science is called Mycology. Mushrooms have considerable interest in the most important civilizations in history because of their sensory characteristics. They have been recognized for their attractive culinary attributes. Presently mushrooms are common valuable foods because they have low calories, carbohydrates, fat, and sodium and are cholesterol-less vegetables. Besides, mushrooms have important nutrients, and are rich in selenium, potassium, riboflavin, niacin, vitamin D, proteins, and fiber. Mushrooms have healing capacities and many properties of traditional medicines. They act as anti-fungal, antibacterial, immune system enhancers and cholesterol-lowering agents, and also are an important source of bioactive compounds. Due to the presence of these properties, a variety of mushroom extracts are used to promote human health and are found as dietary supplements. It has been reported that mushrooms have beneficial effects on health and are a good source of treatment for some diseases and disorders. Some of the nutraceutical properties in mushrooms are seen in the treatment of hypertension, high risk of stroke, Alzheimer's, in reducing the likelihood of cancer invasion and metastasis due to anti-tumoral attributes. Although there are a number of mushroom varieties having nutritional and nutraceutical properties, mostly are collected from nature (wild), whereas a few are cultivated on marginal and commercial label. *Agaricus bisporus*, species of *Lentinula* (*Lentinus*), *Volvariella*, *Pleurotus*, *Calocybe*, *Auricularia*, *Flammulina*, *Ganoderma*, *Schizophyllum*, *Trametes* and few others are among cultivated varieties in India and abroad. Considering the tremendous nutritional and medicinal qualities of different groups of these fleshy fungi, the cultivation of different varieties of mushrooms is increasing globally day by day.

Keywords: Bioactive compounds, Essential amino acids, Human diseases, Medicinal use, Mushroom, Nutraceutical properties, Nutritional value, Vitamins.

* **Corresponding author Diwakar Bahukhandi:** Division of Mycology and Plant Pathology, ICAR-Indian Agricultural Research Institute, Pusa, New Delhi, India; E-mail: diwakar14jan@gmail.com

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INTRODUCTION

Agricultural scientists including phytopathologists normally, looked at fungi as enemies of crop plants which reduce their economic value. Therefore, they concern their efforts to boost farm production quantitatively and qualitatively and concentrate their target on control measures to minimize losses caused by the fungi to crops and thus stabilize food production. On the other hand, the mycologists eagerly try to search the new fungi that can be useful for human beings in multiple ways including their edibility. The fleshy fungi used as food and have nutritious and medicinal properties, are called edible fungi or edible mushrooms. Mushrooms have been considered especially for their unique flavor and have been valued by mankind as a culinary wonder. More than 3,000 species of mushrooms exist in nature globally, but around 40 are widely accepted as food and few are commercially cultivated [1 - 4]. Mushrooms are considered as a delicacy with high nutritional and functional value, and are also accepted as nutraceutical foods. They are of considerable interest because of their organoleptic merit, medicinal properties, and economic significance [5 - 8]. Generally the common edible mushrooms also have medicinal properties [9, 10]. The earliest word for mushroom in Sanskrit appears to be as Ksumpa, Chhatrak, Bhumi kavaka, and in the present day khumbi, dharti ke phul, *etc*., are commonly used for mushrooms in different states/areas. In some places of our country, the term kukurmutta is used for mushrooms or bad mushrooms. The consumption of mushroom as food [11] and drugs [12] goes back in the history of mankind and as old as the civilization itself. References to their occurrence and utilization as food and medicine are found in our classical religious writings like Vedas, Briksha Ayurveda, Chinese literatures and Bible [13]. The importance of kavakas (mushrooms) for nature and mankind and the use of 'Somaras' were authentically described by the great surgeon Shushruta in his famous medical hand out, the Sushruta Samhita. According to medical treatises of India, Samhita of Atreya Charaka period dating back to 3000 (+/-500) BC, the mushrooms were classified into edible, non-edible or poisonous and medicinal [14]. Greeks believed that mushrooms provided strength for warriors in battle, and the Romans perceived them as the 'food of the god' for centuries, whereas Chinese culture has treasured mushrooms as a health food, an 'elixir of life'.

Globally the most commonly cultivated mushroom are species of button mushroom, *Agaricus* [mainly *A. bisporus* (crimini mushrooms, portobello mushrooms) and *A. bitorquis*], followed by shiitake (*Lentinula* or *Lentinus edodes*), paddy straw mushroom (*Volvariella* spp.), oyster mushroom (*Pleurotus* spp.), wood ear or black ear mushroom (*Auricularia*), velvet stem or winter mushroom (*Flammulina velutipes*), black poplar mushroom (*Agrocybe aegerita*) and few others. In India, the cultivation of milky mushroom (*Calocybe indica*) is also getting popularity due to its easy growing technology and high temperature adaptability [15]. A lot of work has been done on different aspects of various mushrooms in different parts of India [16 - 18]. The edible fungi have great nutritional value since they are rich in protein, with an important content of essential amino acids and fiber, poor fat but with excellent important fatty acids content. Moreover, edible mushrooms provide a nutritionally significant content of vitamin B1, B2, B12, C, D, E, *etc* [19, 20]. Globally mushrooms production is continuously increasing, being China the biggest producer, however, wild mushrooms are becoming more important for their nutritional, sensory, and especially pharmacological characteristics [21 - 24]. The mushrooms or macrofungi having medicinal value mostly belong to Basidiomycota and few Ascomycota. Although there a number of other groups of fungi used for medicinal purposes and other effects on human health. There are a number of wild mushroom species, having rich nutraceutical properties, which are not cultivated, because majority of these mushrooms are either mycorrhizal and cultivation technology of others is not known. Species of *Russula*, *Ganoderma* (rishi mushroom), *Grifola* (maitake), *Fomitopsis*, *Phellinus*, *Calvatia gigantea* (giant puffball), *Ramaria* (coral mushroom), *Inonotus obliquus*, *Coriolus versicolor* or *Trametes* (turkey tail fungus), *Schizophyllum commune*, *Cantharellus*, *Laetiporus sulphureus* or chicken of the woods or sulfur mushroom, *Laccaria*, *Craterellus*, *Eutypella*, *Morchella* (Morels), *Tuber* spp. (truffles), *Hydnum* spp. (hedgehog), *Lactarius* (milk mushroom), *Hericium erinaceus* (Lion's Mane), *Boletus edulis* (king bolete**)**, *Tremella fuciformis* (snow fungus), *Termitomyces* (termite mushrooms), *etc*., are the mushrooms of economic importance. *Penicillium*, *Ustilago maydis* (corn smut), and various other fungi are important for their medicinal, nutritional, biocontrol, and industrial importance. Mushrooms are a complete food as well as possess nutraceutical properties and are directly consumed by humans.

In literature, more than 130 medicinal functions are mentioned that are produced by fungi including mushrooms and the key medicinal uses are antioxidant, anticancer, antidiabetic, antiallergic, immunomodulating, cardiovascular protector, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxification, and hepatoprotective effects [3, 25]. Numerous molecules synthesized by macrofungi are known to be bioactive compounds, which are found in fruit bodies, cultured mycelium, and cultured broth are polysaccharides, proteins, fats, minerals, glycosides, alkaloids, volatile oils, terpenoids, tocopherols, phenolics, flavonoids, carotenoids, folates, lectins, enzymes, ascorbic, and organic acids [26]. Polysaccharides are the most important for modern medicine and beta-glucan is the best known and the most versatile metabolite with a wide spectrum of biological activities [23, 27]. A balanced diet is the supporting treatment for the prevention of illness and especially against

CHAPTER 18

NPs and Soil Microorganisms Interactions in Crop Management - Current Status and Future Prospects

F. Ahmad[1] and **S. Ahmad**[1,*]

[1] *Eros Garden, Charmwood Village, Faridabad, Haryana, India*

Abstract: Size and surface modifications of a nanoparticle (NP) make it easy to cross several physiological barriers and mix with the transport of numerous bio-actives not only to selectively interact with different molecular species but also adopt characteristic pathways depending upon their physicochemical properties. Successful realizations of these possibilities associated with the development of biomedicines have already been realized in several cases published recently. Drawing a parallel from these observations the next question is whether similar possibilities can be availed in the case of agricultural crop management, especially with an eye on improving crop health to meet the global need for food security without any adverse effect on the natural ecological balance. The interaction space of nanoparticulate species, prepared separately, is more heterogeneously complex due to additional contributions from the ecosystem.

For appreciating the numerous advantageous applications of the NPs in crop health management, first, it is necessary to know about the constituents of the soil including bio-organisms that facilitate supplying adequate micronutrients to the plants through their roots along with the coexistence of various families of fungi and pathogenic species. In this chapter, an attempt has been made to examine the interactions of numerous types of metal-NPs on the populations of fungi and bacteria at molecular levels for using the relevant interactions to improve plant health, growth, and yield with adequate protections from harmful species also present there. The experimental assays made *ex-situ* and *in-situ* in simulated models as well as actual cases of different crops are included in the descriptions to provide a more integrated understanding of the interactions involved. The contributions from very recent reviews already published are acknowledged duly for providing input for the discussions regarding the prospects.

Keywords: Arbuscular mycorrhizal fungi, Bacteria, Interaction, Nanoparticles, Plants.

[*] **Corresponding author S. Ahmad:** Eros Garden, Charmwood Village, Faridabad, Haryana, India; E-mail: drsahmad@email.com

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INTRODUCTION

While examining the influence of nanoparticulate material species on overall crop management, it is advisable to look at the whole situation in an integrated manner involving components like nanoparticulate material species interacting with soil microbes (symbiosis of pathogens and fungi) present in the proximity of the plant roots along with the secretion of bioactive compounds influencing the plant health genetically in the presence of the prevailing environmental conditions. In the absence of this holistic approach, perhaps, the experimental results derived from different assays might offer contradictory views. Interactions taking place with nanoparticulate species also differ considerably because of their surface modifications arising out of different biochemical pathways followed while entering the plant roots in the presence of a large variety of biochemically active compounds derived from different resources of microorganisms including fungi and microorganisms that are specific to the location, weather, and environmental condition.

One of the main reasons to understand the basic processes involved in NP-interaction is the major concern arising out of the adverse effects of consuming several types of chemical fertilizers and pesticides on the environment, human health, and microbial properties of the soil that can hardly be ignored especially in industrial agriculture for meeting global food security, particularly, when sustainable agriculture is the 'global priority'. Consequently, agricultural crop management has already started putting pressure on examining the other alternatives to take care of these ensuing problems internally (fungal, and bacterial populations) and externally (exposure to nanoparticulate material species). In this context, extensive efforts are currently going on to examine arbuscular mycorrhizal fungi (AMF) for improving plant growth and conferring mycorrhiza-induced resistance (MIR) *via* several mechanisms inducing defense compounds, and sensitization of the plant's immune system for creating more protection against later arriving pests or pathogens signaled through jasmonic acid (JA). The environmental conditions, of course, do control the plant growth along with the resistance derived from AMF. Low soil P and unlimited light conditions favoring AMF colonization due to positive crosstalk between the plant's phosphate starvation response (PSR) and JA-dependent immunity are expected to enhance MIR. The growth and resistance benefits were assessed in case of AMF *Funneliformis mosseae* in *Plantago lanceolata* plants grown under different levels of soil P and light intensity. Resistance benefits assessed in the case of the leaf-chewing herbivore *Mamestra brassicae* clarified that half of the plants had jasmonic acid induced prior to the bioassays to test whether AMF primed plants for JA-signaled defense under different abiotic conditions. Reduced biomass production due to AMF was not the strongest under conditions considered least optimal for carbon-for-nutrient trade (low light, and high soil P). Although JA-applications induced resistance to *M. brassicae*, but its influence was independent of soil P and light conditions. In younger plants, JA-induced resistance was annulled by AMF under high soil P, and ample light showed that AMF repressed JA-induced defense responses. In older plants, low soil P and light could enhance the susceptibility to *M. brassicae* due to enhanced leaf N-levels and reduced leaf levels of the defense metabolite catalpol contrary to younger plants. The defense priming by AMF is not ubiquitous and calls for further studies to reveal the causes of the increasingly observed repression of JA-mediated defense by AMF as discussed recently [1].

Soil microorganisms participate in immobilizing carbon, nutrient cycling, and detoxification *via* contaminant degradations leading to better soil properties. Almost 15% of the total populations of soil microbes belong to bacterial species, known as plant growth-promoting rhizobacteria (PGPR) that form colonies in the plant roots and influence directly/indirectly the plant growth. Out of the large PGPR family comprising *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Bacillus*, *Thiobacillus*, *Pseudomonas*, *Azospirillum*, *Burkholderia*, *Arthrobacter*, *Acinetobacter*, *Agrobacterium*, *Serratia*, and many others; only 2-5% of rhizosphere bacteria are of potential uses [2 - 5].

Many microorganisms while interacting with the plants in the rhizosphere form mutually beneficial alliances in the natural ecosystem. AMF, while establishing a symbiotic association with the plant roots receiving photosynthetic products, helps in improving nutrient uptake, enhancing biomass accumulation, and improving photosynthesis in 80% of the terrestrial plant roots, where fungal hyphae percolate in the cortical cells of plant roots forming arbuscules, vesicles and hyphae. They help in binding heavy metal (HM)-based contaminants at the cortical region of the roots by binding and preventing their translocation towards aerial parts of the plant preventing damage to leaf-tissues. AMF promotes plant growth by ameliorating water uptake and controlling stomatal conductance. AMF provides one of the best biological methods for enhancing plant growth and shoot biomass while detoxifying HMs by immobilizing them in fungal structures *via* precipitation and chelation, sequestration in vacuoles, and activation of the antioxidants. Maintaining AMF is an eco-friendly way of acquiring sustainable productivity by improving soil health and protecting plants against abiotic and biotic stresses as reported in the published literature [6 - 10].

The soil-borne microorganisms help in improving plant nutrient uptakes and introducing resistance to several abiotic stresses. Most of the AMF species belong to the sub-phylum Glomeromycotina of the phylum Mucoromycota. Four kinds of AMF namely: Glomerales, Archaeosporales, Paraglomerales, and Diversisporales,

CHAPTER 19

A Synergistic Metagenomics Approach to Bioino-culants

Ashwini A. Waoo[1,*]

[1] *Department of Biotechnology, AKS University, Satna – 485001, MP, India*

Abstract: Sustainable farming is an emerging trend in recent decades to improve ecosystem health. However, little is known about to what extent and how this process affects the taxonomic diversity and functional capacities of above-ground microbes. Consequently, a metagenomics approach was applied to investigate how agricultural management practices, including organic, and conventional management, govern the structure and function of soil microbial communities. In a metagenome analysis, farming practices are strongly influenced by taxonomic and functional microbial diversity, and interactions of microbes. In agricultural soil, the most complex microbial network was observed that can be used for bioinoculant production and their applications for bioremediation of contaminated agricultural soil, indicating a strong resilience of the microbial community to withstand environmental stresses.

The metagenomics of soils can provide an assessment of the largely untapped genetic resources of soil microbial communities independent of cultivation for bioinoculant production. Novel biomolecules and genes have been identified by this approach. It also helps to study the metabolism of microorganisms that change in response to different environmental conditions. This chapter describes the use of these novel tools in the exploration of soil microbiota and its use to innovate new farming practices for a sustainable environment.

Soil microbial communities are the most complex of any other microbial communities. Methods based on sequencing remain the most effective way to analyze soil metagenomes. Future strategies to overcome this difficulty include comparative sequence analysis using soil metagenome sequences to identify microbial enzymes and novel bioactivities. The Metagenomics approach provides benefits over the restrictions of culture-dependent procedures along with the study of the community structure and function of microbes in the soil.

Keywords: Agriculture, Bioinoculants, Bioremediation, Microbes, Metabolism, Soil.

* **Corresponding author Ashwini A. Waoo:** Department of Biotechnology, AKS University, Satna – 485001, MP, India; E-mail:ashwiniwaoo@gmail.com

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INTRODUCTION

Conventional agriculture negatively impacts the environment, *e.g*., by depleting soil microbial diversity, soil quality, drinking water supply, and plant productivity. The use of sustainable agricultural technologies, such as biofertilizers, can reduce the use of chemical fertilizers and the detrimental effects of agriculture on the environment. Chemical fertilizers are often applied excessively and continuously, compromising the chemical and biological properties of soils, causing pollution of ecosystems, and harming human health. Kopittke and coworkers [1] state that nutrient and biodiversity depletion lead quite frequently to the chemical and biological degradation of agricultural soils. As a result, such inadequate soil fertility often requires the use of chemical fertilizers and pesticides, which in turn can increase the cost of agricultural management. The quest for more sustainable agricultural practices in the current scenario has focused attention on the biological potential of the interaction between crops and microorganisms dwelling in rhizospheres [2].

Agronomic fortunes have shifted due to bioinoculants nowadays. Despite its success in developed countries, the exploitation of bioinoculants is hindered in developing countries. The use of bioinoculants will be successful with scientific knowledge and its application. However, neglecting to ensure and maintain a high-quality standard of the product will have negative consequences. By understanding how bioinoculants work, better tagging of resources can be achieved. Hence, the chapter provides an overview of different bacteria-derived, fungal-derived and algal biofertilizers, their associations with plants, and the transformation of nutrients in the soil. In developing sustainable agriculture, a rational approach to the use and management of microbial fertilizers is vital.

Metagenomics, the process of isolating and testing clone libraries derived from soil DNA, offers the possibility of determining the genetic composition of soil communities independent of cultivation. A novel gene and biomolecule have already been identified using this method. The metabolic foot printing of different microorganisms that are exposed to different environmental conditions can be used to study their metabolism. Temperature, pH, and nutrient concentration influence the concentration of extracellular metabolites. Metabolite uptake and secretion from the soil are affected by these factors. Using these novel tools, this chapter shows how soil microbiota can help us innovate new agricultural norms for ensuring sustainability [3].

Identification of these novel genes can be done using soil microbes. In metagenomics, the complete soil microbial diversity is accessed, no matter whether it is cultivable or uncultivable. For gene identification, recent molecular methods include direct genomic DNA extraction, metagenomics libraries, heterologous gene expression, and high-throughput next-generation sequencing. Sustainable agriculture requires frequent exploration and characterization of PGPRs. Despite the culturable subsets, metagenomics can yield a substantial amount of genetic information. Moreover, it is also potentially useful for bridging gaps in the genetic evolution of microbial communities of different unknown species. Next-Generation Sequencing (NGS) technology made a combination of high-throughput metagenomic analysis, crop genotyping, and the detection of plant pathogens and PGPRs.

Microbial inoculants are critically important for the development of sustainable agriculture if we understand the mechanisms of action they employ. We can eliminate any chemicals from our diets if we avoid using them in agriculture. Microbial inoculants can be used as bio-control agents and bio-herbicides to control pests and weeds. A sustainable approach to improving farm productivity and food quality involves utilizing natural resources such as beneficiary microorganisms.

SOIL HEALTH MANAGEMENT

Physico-chemical constituents and organisms in soil are interconnected, allowing the soil to be a self-organizing system. Yet, even though microbes are extremely adaptable, changing climates and land management also affect them. A soil's resistance refers to its ability to maintain itself no matter how large the changes caused by any perturbation may be. Resilience is the ability of the soil to return to its original state following a disturbance also known as its self-healing capacity [4].

Due to its diversity of microbial species, the soil is a dynamic ecological habitat for study due to the vast number of species present there. Several unknown functions in soil are critical to life's sustenance. Due to advances in agriculture techniques and management practices, recent technologies have intensified agriculture without greatly enriching the soil. As a result of this overuse, the soil structure and fertility of the cultivable farmlands have deteriorated as well as the ability to sustain life. Many cultivable areas have become saline or uncultivable.

Traditional agriculture and its practices negatively affect soil vitality, altering microbial diversity, and degrading the global food chain and food safety. Chemical pesticides applied excessively and sometimes inappropriately in agriculture have resulted in agroecosystems degradation and pollution. They have polluted water reservoirs and contaminated soils, all of which have subsequently led to biodiversity loss by killing plants, animals, insects, aquatic ecosystems, and wildlife, and poisoning farm workers. Furthermore, intensive agriculture under

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Shampi Jain

Ms. Shampi Jain, an agricultural economist is presently working as an Assistant Professor, at the Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, India. She completed graduate and postgraduate from Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalay; Chitrakoot, Satna, Madhya Pradesh, India. She is a gold medalist in postgraduate. She has a more than 3 years of teaching experience and has published research papers and written several articles for newspaper related to Agriculture Economics. She has attended four national and one International conference and three workshops related to her field. She has two authored books on her credit. She is also a member of professional societies related to Agricultural Economics. She is working on various aspects of rice and wheat economics and the impact of diseases on the economy of various leguminous crops.

Ashutosh Gupta

Ashutosh Gupta is working as an Assistant Professor, the Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, India. He has obtained his post-graduation in Agriculture Soil Science and Agriculture Chemistry from the College of Agriculture, C.S. Azad University, Kanpur, India. He has more than Five years of teaching and research experience in soil science, agriculture microbiology, soil chemistry, plant-microbe-soil interactions as well as in environmental sciences. He is the recipient of state fellowships at the national level. He is currently engaged in research on soil microbes and their characteristics in Indian soil.

Neeraj Verma

Prof. Dr. Neeraj Verma, Ph.D. (Botany) with specialization in Botany (Plant Pathology) is working as an Associate Professor, the Department of Agriculture Science, Faculty of Agriculture Science and Technology, AKS University, Satna, India since 2012. Earlier he was an Assistant Specialist (Plant Virology), at the University of California Riverside, Riverside, CA, USA. He has research and teaching experience of more than 20 years. He has published more than 45 International and National research papers and book chapters and has published two books. He has worked on various research projects funded by government agencies. He has attended many national and International conferences. He is an editor and reviewer of several professional journals.