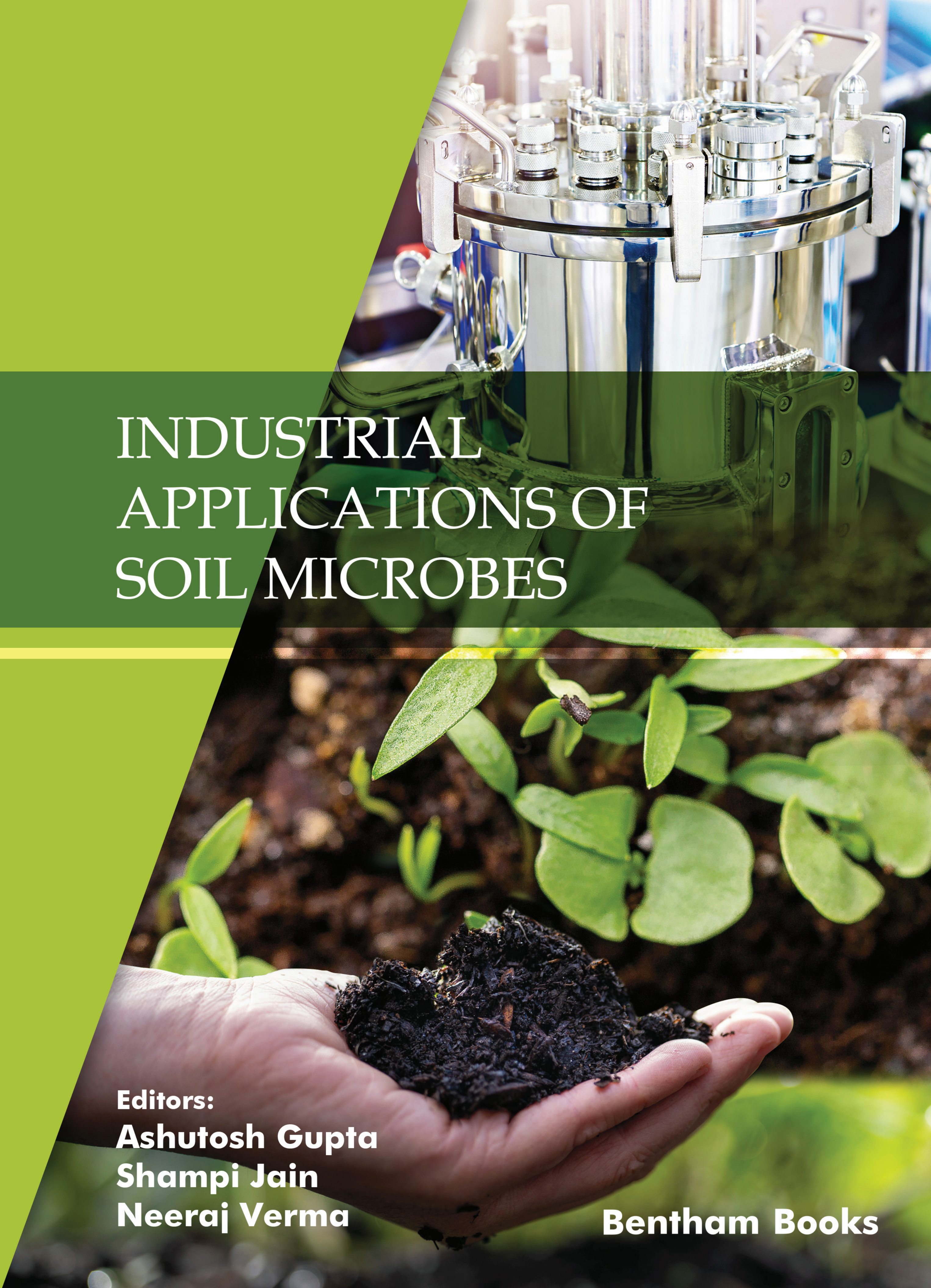
INDUSTRIAL APPLICATIONS OF SOIL MICROBES



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Industrial Applications of Soil Microbes

(Volume 3)

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FOREWORD

A holistic approach is particularly needed to understand soil microbes and their products, which secrete or change the chemicals present in the soil by their biological processes. These soil microorganisms are not only directly or indirectly influenced by environmental conditions, fundamental soil characteristics such as moisture, oxygen and chemistry and also by each other in both beneficial and predatory ways. By studying all components in all-inclusive means, we can learn how to manage soil in a way that enhances the benefits provided by soil organisms and, ultimately enhances the fertility of the soil.

The soil microorganisms live on organic matter, make an association with other organisms and perform significant vital processes in the soil. Of these microorganisms, bacteria, fungi, algae and nematodes are the most important ones. Some of them perform critical functions in nitrogen and carbon cycles. Mostly the soil organisms are friendly except for a few. These microbes carried out various ecological processes, necessarily not only essential for plants but significant for animals and humans. They also carried out the degradation of organic material and suppressed soil-borne plant diseases.

The present book "Industrial Applications of Soil Microbes" Vol 3 presents the basic information about these microbes, especially on mycorrhiza and soil-borne bacteria. The different chapters written by eminent subject experts will not only provide the basics about soil microbes but also their products, which are of importance to humankind. I am sure that the knowledge given in the form of the book will be not only helpful to the students but also to the scientists, academicians and industrialists. I, personally hope that the book will be helpful in giving an idea for the future direction of research.

I congratulate all the contributors of this book and the editorial board members for bringing up such an important book for microbiologists.

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PREFACE

Soil is a home of various living microorganisms, such as algae, fungi, bacteria, nematodes, *etc*., that live and carry out their activities. A handful of soil contains billions of bacteria, fungi and other microorganisms. They play important roles in the nutrient cycle and make the availability of nutrients to plants. These microorganisms also degrade the dead and decaying parts of plants and animals that ultimately break up into minerals and make the soil healthy and fertile for crops to be grown there. These microorganisms not only interact with each other but also with the rhizosphere of the plant. The plant root exudates also influence the population of microorganisms.

Some microorganisms are found to be associated with particular plants and help those plants specifically. Microorganisms secrete chemicals or metabolites such as enzymes, which are helpful to plants and mankind in several ways. They may help plants to reduce stress and also increase the metabolic reactions to produce specific chemicals that may help for industrial purposes. These chemicals, secreted by microorganisms of plants in the presence of microorganisms, may be used by industries like pharmacy, leather, soaps, food, agriproducts, *etc*.

In the present volume 3 of the book, emphasis is being given to various soil microorganisms, including bacteria and mycorrhiza. The chapters contributed by eminent scientists and researchers have emphasized not only the introduction of these microorganisms but also their industrial uses as well as the effect on the plants. The chapters are written to provide information to the graduate and research students and for the scientists and academicians to get up-to-date knowledge of the subject. The authors have tried to provide the recent developments in the subject.

With positive hope, the editors of the book will be happy to receive suggestions and comments to improve the information for further editions.

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CHAPTER 1

Mycorrhiza and its Applications in Agriculture and Forestry

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Abstract: The symbiotic association between green plants and fungi is called mycorrhiza. The plant makes organic products by photosynthesis and supplies them to the fungus, and the fungus from the soil supplies water and mineral nutrients, such as phosphorus, *etc.*, to the plant. These fungi establish a mild form of parasitism, a form of mutualism, where both the plant and the fungus benefit from the association. Mycorrhizal fungi are soil fungi that play an important role in plant growth, protection of plants from pathogens, and improving the quality of the soil. Abiotic components and living communities of soil and soil organisms, particularly microbes, can have direct and indirect impacts on land productivity. Direct impacts are those where specific organisms affect the crop yield immediately. Indirect impacts that affect the functions include those provided by soil organisms participating in carbon and nutrient cycles, soil structure modification, and food web interactions that generate ecosystem services that ultimately affect plant productivity. Selected organisms from different functional groups, like microsymbionts (symbiotic fungi, bacteria, *etc.*), decomposers, elemental transformers, soil ecosystem engineers, soil-borne pests and pathogens, and micro regulators, are used to illustrate the linkages between soil biota and ecosystem processes. There are various groups of fungi that form different types of symbiotic associations with almost all groups of plants, from bryophytes to seed plants, *i.e.*, gymnosperms and angiosperms, on the earth. Out of the seven types of mycorrhizae (ectomycorrhizae, ectendomycorrhizae, ericoid mycorrhizae, arbuscular mycorrhizae, orchidoid mycorrhizae, arbutoid mycorrhizae, and monotropoid mycorrhizae), the endomycorrhizae (arbuscular) and ectomycorrhizae are the most abundant and widespread. The molecular basis of nutrient exchange between ectomycorrhizal and arbuscular mycorrhizal fungi and host plants proved the role of mycorrhizal fungi in disease control, the alleviation of heavy metal stress, and increasing production in sustainable agriculture, horticulture, and forest plants or trees, *etc.* Arbuscular mycorrhizal fungi play a major role in the restoration of native ecosystems, and mycorrhizae transform a disturbed ecosystem into productive land. Ectomycorrhizae play an important role in forestation, forest ecosystems, and horticultural systems, and they maintain monodominance in tropical rainforests. Apart from the nutrient benefits to the plants,the mycorrhizae are presently employed in the colonization of barren soil

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and improving the transplantability of forest plants. Mycorrhizae create resistance against insect pests, various root diseases, toxicity, and reduced susceptibility in plants. The presence of mycorrhizae also favours the growth of beneficial microbiota, converting the rhizosphere into a mycorrhizosphere and increasing tolerance to adverse conditions like drought, salinity, and stress in the plants.

Keywords: Agriculture, Disease Resistance, Drought Stress, Forestry, Fungal Biodiversity, Host Plant Interactions, Mycorrhizae, Mineral Uptake, Rhizosphere, Saline Tolerance, Soil Components, Symbiotic Association.

INTRODUCTION (SOIL, PLANTS, AND MICROBES)

Soil and the Environment

Soil is the outermost layer of the lithosphere and the key element of the environment on which human beings depend for their existence. Soil is the birthplace of microbes, including fungi, plants, and varieties of organisms, and is a medium for the circulation of nutrients, biotic, and abiotic processes. Several physical and other parameters, such as moisture, pH, temperature, seasonal variation, nitrogen, phosphorus, organic matters, and types of soil and their texture, influence the occurrence, distribution, and quantitative and qualitative accounts of fungi and other microbes. The soil is a homogeneous system of solid, liquid, and gaseous constituents formed by the decomposition of plant and animal remains [1]. Soil is broadly divided into mineral particles, plant and animal residues, living systems, water and gases. The soil strata contain undecayed or partially decomposed litter on the top and unweathered parent rock at the bottom. The upper or top layer, considered the fertile zone of soil (6-10 cm), contains mineral and organic matter and is of high microbial activity. Soil organisms are responsible for carrying out many vital functions as they anchor the plant roots, provide air, and supply water and nutrients for their growth and development. The ecosystem capable of producing the resources necessary for the development of living organisms in soil is called the “soil environment”. Soil can self-produce resources necessary for the development of living organisms, which makes it a living environment for various species existing on earth. This component is known as the “earth’s biological engine”. Soil quality can be defined as the balance between high activity and high microbial diversity, which plays an important role in protecting the environment, preserving biodiversity, and improving agricultural practices. Agriculture has an impact on soil and, thus, on the qualitative and quantitative composition of soil microorganisms.

Soil Organisms and Microbes

Soil organisms are organisms that inhabit the soil during all or part of their lives. They range in size from microscopic cells and protozoa to amphibians, reptiles, and small mammals and play an important role in maintaining fertility, structure, drainage, and aeration of the soil. Some soil organisms are pests of crops, such as nematodes, slugs and snails, symphylids, beetle larvae, fly larvae, caterpillars, and root aphids, whereas others are pathogenic and cause plant diseases such as anthracnose and rot, and still, others are hosts for organisms that cause animal diseases. It is estimated that one square metre of rich soil can harbour as many as one billion organisms [2]. The prime organisms are protists, microfauna, mesofauna, macrofauna, and megafauna. In agricultural systems, three main groups of biogenic structures are found: earthworm casts, termite mounds, and ant heaps. The biogenic structures can be deposited on the soil surface or in the soil, and they have different physical and chemical properties from the surrounding soil.

Soil microorganisms are unicellular or multicellular organisms that constitute the highest biomass. They include actinomycetes, cyanobacteria, bacteria, algae, fungi, yeasts, myxomycetes, viruses, protozoa, mites, nematodes, *etc.* They are responsible for biomass decomposition, circulation of the biogenic elements that make nutrients available to plants, biodegradation of impurities, and maintenance of soil structure. They are abundant near plant roots, one of their food resources, and are involved in many biogeochemical processes like mineralization of organic matter, element circulation, synthesis of proteins and nucleic acids, as well as the transformation of phosphorus forms [3, 4]. Soil fungi are responsible for decomposition in terrestrial ecosystems as they degrade and assimilate cellulose and lignin [5]. It is estimated that soil microflora performs 60–80 percent of total soil metabolism. One gram of topsoil may contain approximately 1 billion bacteria, 100 million actinomycetes, 1 million fungi, and 100 nematodes. Microbes, including fungi, participate in different important nutrient cycles in nature, such as the sulphur cycle, carbon cycle, nitrogen cycle, phosphorus cycle, *etc.*, to maintain a balance of the soil environment.

Soil-Plant-Microbe Interrelationships

The addition of organic matter is one of the various important methods employed for conserving the land from soil erosion, which improves the physical and biological properties of the soil and reduces water and soil losses. This improves the physical properties of the soil and encourages microbial activity and humus formation. It is well known that organic matter, such as polysaccharides, lignin, *etc.*, binds soil particles, and mycelial strands of fungi, actinomycetes, and the

CHAPTER 2

Mycorrhiza-The Multifunctional Biofertilizer

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Abstract: The role of fungi, particularly those selectively colonizing the root surfaces of growing plants, in increasing the availability of phosphorus has received scanty attention. The fungi arbuscular mycorrhiza (AM) has many beneficial effects on plant growth, including enhanced nutrition, improved plant growth, and better biotic and abiotic stress tolerance. Moreover, by improving the soil properties, the hyphal networks of these AM fungi will minimize the risk of water and wind erosion. This potential of AM fungi encourages a flourishing industry of AM-related substrates, mainly in the plant production and landscaping sectors. Although the potential benefits of AM fungi for some crops have been well documented, more research is needed to determine their suitability for other crops. The various aspects, which are fully reflected in this chapter, are mycorrhiza-history, classification, mode of action, crop specificity, AM production technology, quality standards, and methods of analysis, along with future opportunities for AM application.

Keywords: Arbuscular Mycorrhiza, Colonization, Growth, Landscaping, Nutrition.

INTRODUCTION

Mycorrhiza exhibits a symbiotic relationship with plant roots by facilitating the plant's uptake of nutrients and water from the soil. In exchange, the fungi are supplemented with food (in the form of carbohydrates) from the host plant. These organisms are known to not only mobilize phosphates but also perform the function of transferring phosphorus from the soil into roots. Unlike nitrogen-fixing and phosphorus solubilizing biofertilizers, mass production of AM fungi is considered difficult, which restricts their practical application. AM fungi are virtually ubiquitous, being present in tropical, temperate, and arctic regions. These fungi usually exist in natural habitats and provide a wide range of ecological services like improved soil structure, enhanced stress tolerance, and plant nutrient

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availability. As these organisms symbiose with many plants (cereals, fruit crops, and vegetables), interest in their potential applications towards sustainable agriculture is noticed.

History

The name mycorrhizae originated from the Greek words "myco" and "rhiza," which mean fungus and root, respectively. Despite its occurrence in the plant kingdom revealed a long time ago, the benefits of this fungus in improving plant nutrient absorption and translocation have drawn the scientific community's attention only in the recent past. AB Frank was the first person to draw attention to this unique symbiosis between plant roots and fungi.

Classification

Based on morphological, structural and functional aspects, there are mainly two types of mycorrhiza: (i) Endomycorrhiza or AM mycorrhiza, or arbuscular mycorrhiza (formerly termed vesicular-arbuscular mycorrhiza or VAM) and (ii) Ectomycorrhiza.

Other groups that are also ecologically significant are ericoid mycorrhiza (ErM) and orchid mycorrhiza (OM). Ericoid mycorrhiza (ErM) is specific to ericaceous plants, which generally grow in stressed environments where extreme soil acidity and low rates of nutrients exist. Orchid mycorrhiza (OM) is generally associated with orchids.

Endomycorrhiza

Arbuscular mycorrhiza (AM mycorrhiza or VAM) is the most important type of endomycorrhiza present in the roots of most vascular plants. These fungi belong to Zygomycetes, a family of Endogonaceae of the order Glomales (phylum Glomeromycota). These fungi are distinguished by their perforation into root cells (intracellular) and the development of unique structures called arbuscules.

Ectomycorrhiza

This is the most characteristic mycorrhiza of forest trees. It belongs to the Basidiomycetes, mostly to the species of *Amanita*, *Boletus*, *Cortinarius*, *etc*. Ectomycorrhiza is formed by about 3% of plant species. These are the intercellular organisms where the fungi are attached to the outer root surface (external mycorrhiza). In ectomycorrhiza, specific structures are formed during symbiosis. These are the hyphal mantle, the Hartig net, and the external mycelium.

FUNCTIONAL SPECIFICITY OF AM TO CROP PLANTS

Mycorrhizae possess a mutualistic relationship with many plants (around 80% of vascular plants) by assisting them in nutrient mobilization, and in return, AM gains photosynthates from the plants. However, the effect of AM on different plant species is not uniform and hence sometimes results in neutral or negative impacts. Contrarily, the sporulation and penetration of AM fungi depend highly on their host plant suitability [1].

Favourability of AM

Despite the wide applications in agriculture and horticulture, the effectiveness of AM fungi in field conditions depends largely on the external environment. For example, practices like ploughing and excessive application of fertilizers (mainly P) restrict the establishment and colonization of fungi [2, 3]. Other factors that influence AM abundance include soil conditions, the dominance of other microorganisms, planting non-host species (plants that belong to Brassicaceae and Chenopodiaceae), and using certain biocides [4].

The benefits associated with fungi-plant combinations rely mostly on existing environmental conditions. Under the prevalent situations of abiotic stresses and nutrient deficiencies, AM symbiotic plants were found to have superior growth over non-mycorrhizal species. Thus, mycorrhizae contribute to a potential intra-specific competitiveness advantage and favours the associated plant species.

APPLICATION TECHNOLOGY FOR MYCORRHIZA (AM)

Two methods are important, viz., nursery application and polybag application [5].

Nursery Application

A bulk inoculum of 100 g is required for a one-meter square nursery. Inoculums are applied to the soil at 2-3 cm depth at the time of planting or sowing of seeds. Care should be taken to plant/sow the seeds above the AM inoculums so that the root coverage from the above material may contact the inoculum and cause infection.

Poly Bag Application

It can be used for forest trees, coffee, tea, *etc.*, at their seedling stage. In general, 5-10 g of bulk inoculum is required for one polybag. The polybag consists of the potting mixture (10 kg of inoculum and 1000 kg of sand potting mixture).

CHAPTER 3

Soil Mycorrhizae and Their Industrial Applications

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Abstract: Over the past few decades, the growing body of research on mycorrhizal fungi has been exploring their roles in maintaining and enhancing a wide range of ecosystem functions. These functions include, and are not limited to, maintenance of soil health, plant nutrition, removing hazardous contaminants from soil, prevention of soil erosion, and suppressing pathogens in the soil. As a result, mycorrhizae offer great potential as ecosystem engineers, capable of meeting various objectives of sustainable agriculture, forestry, ecological restoration, and biodiversity conservation. In this chapter, we attempt to offer an insight into the fascinating world of such mutualistic interaction, some of the benefits it offers to our planet, some of its industrial applications, and why it is imperative to integrate mycorrhizae into discussions for a more sustainable future. We consider various types of mycorrhizae present in our ecosystems and their defining features and differences. After all, we discuss some of the major roles they play in ecosystem functioning. We then explore a few facets of their industrial importance in biofertilization and phytoremediation, which are increasingly recognized globally. We also discuss the issues that hinder the full-fledged utilization of such a mutualistic interaction. In conclusion, we will look at new avenues of research that mycorrhizal research is poised to explore. This chapter will give the readers a holistic view of the exciting world of plant-fungal mutualism and trigger them to explore the growing body of work probing into such fascinating members of our ecosystems.

Keywords: Ecological Restoration, Mutualism, Mycorrhizal Technology, Soil Fertility, Symbiosis.

INTRODUCTION

We often do not realize how mycorrhizal associations have shaped our ecosystems. It is believed that when the earliest plants started their transition from an aquatic environment to the land surface around 500 million years ago, those plants (with a very rudimentary root system) were surely not equipped to extract nutrients for their growth from the mineral soils around them. Perhaps a symbiotic

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relationship developed between plant roots and soil fungi that enabled the plants to derive nutrients from the soil, facilitating their colonization of land that eventually led to the evolution of terrestrial plants [1]. Frank [2] was the first to identify the widespread nature of these soil microbial-plant relations, and it is this relationship that he termed 'mycorrhiza' (taken from the Greek words "*mykes*" meaning fungus and "*rhiza*" meaning root, plural-mycorrhizae). He also correctly hypothesized that the relationship is a case of mutualism, in which the fungi and the plant host nutritionally rely on each other. The association of mycorrhizal fungi with plant roots is perhaps the oldest and ecologically most important symbiotic relationship.

[**N.B.**: Before we proceed further, it would be helpful to keep in mind the difference between 'symbiosis' and 'mutualism'. In common usage, these two terms have often been used interchangeably. However, for this chapter, let us follow the more robust scientific definition of these terms. Symbiosis refers to a close and prolonged association between two organisms of the same or different species [3]. Mutualism means mutually beneficial interactions between members of the same or different species. From this, we can infer that mutualism falls under the broader umbrella term of symbiosis. Symbiosis also includes parasitism, commensalism, and amensalism. The interactions between plant roots and associated fungi do cover all these categories (Fig. **1**). However, by convention, only when this relationship is mutualistic (beneficial for both partners), we term it 'mycorrhiza'].

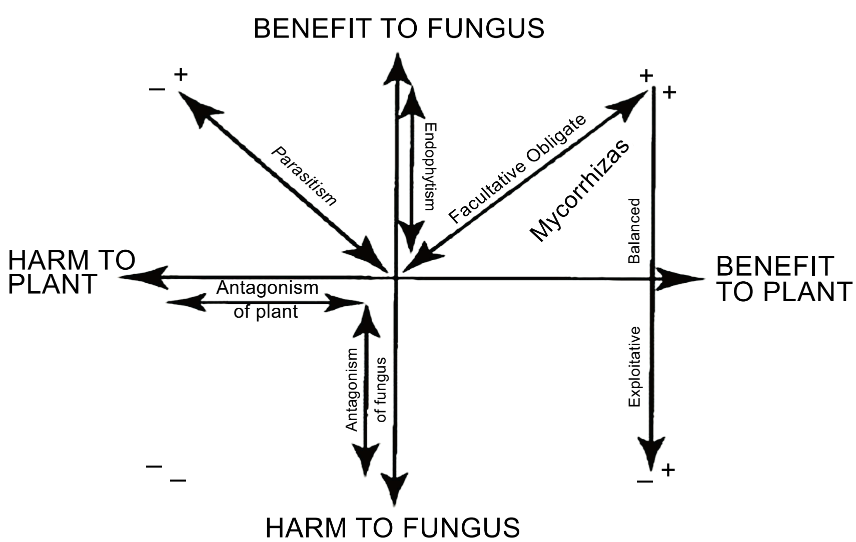

Fig. (1). Plant-fungal interaction categories [4].

Fungi, in general, form a crucial component of the soil microbiome (the aggregation of all microbes in soil) because they decompose organic matter and wood components like lignin in soil. These saprophytic fungi make up the bulk of the fungal biomass present in the soil. The mycorrhizal fungi, which act as nutrient exchange centers in the soil, remain attached to the plant roots and are not found freely in the soil. The body of a fungus is made up of branched threads called hyphae, which intermingle to form a dense, complex network of mycelium (plural-mycelia). In undisturbed soil, a single fungus' mycelium can stretch very long distances (in the case of old-growth forests, it can stretch up to a few kilometers) and thus cover a vast volume of soil. Therefore, mycorrhizal associations give plants considerable benefit by working as extensions of the root system, scouring for resources from bigger volumes of soil and thus expanding the rhizosphere of the plant.

Almost 90% of the terrestrial plant families exhibit associations with mycorrhizal fungi. The fungal partner in this association is an obligate symbiont that depends on the plant for fixed carbon for its nutrition. The life span of the mycorrhizal fungi is determined by their performance and ability to deliver nutrients to the host plants and meet their nutritional demand. If such a partnership becomes inefficient, then other more efficient fungi can be recruited by the plant to carry out its role [5]. All these characteristics make mycorrhizae (and many such symbiotic relationships) appear like a thriving market economy (in fact, a formidable amount of research is going on to explain biological relationships by using market theory and economics for robust understanding [6]. Mycorrhizal fungi exist inside the cortex of plant roots, on the surface of the roots, or around the root epidermis. The benefits received by the plant include improved water relations and resistance to pests and diseases. However, the most important benefit for the plant (which will be discussed in more detail later) is the increase in the uptake of nutrients like phosphorus and zinc from the soil, which are essential nutrients for plants but are rather immobile in soil. Therefore, mycorrhiza is a prominent example of mutualism, much like the root nodule bacteria within legumes. All these functions make mycorrhizal symbiosis exert a strong influence on plant productivity, fitness, soil health, and ecosystem functioning. The key interest of biologists and ecologists is to understand the role of mycorrhizae in resource partitioning and exchange, plant-plant below ground communication, and global nutrient cycling.

TYPES OF MYCORRHIZAE

Depending upon the kind of partners and the structure of the bonding between them, mycorrhizae have been divided into a few major categories (Table **1**) like arbuscular, ecto-, ectendo-, ericoid, and orchidaceous mycorrhizae (Fig. **2**).

CHAPTER 4

Herbicide Effects on Arbuscular Mycorrhizal Fungi and their Symbiosis with Weeds and Crop Plants

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Abstract: Weeds are a serious problem in agriculture, causing major losses in crop production. Chemical methods for weed control, including herbicide use, may have a harmful impact not only on untargeted plants but also on other beneficial organisms, such as arbuscular mycorrhizal fungi (AMF), which form with plant roots, one of the most widespread symbioses on Earth. AMF forms a profuse mycorrhizal mycelial network that explores and scavenges the soil for nutrients and water and links neighbouring plants, thus supporting the transfer of nutrients from one plant to another.

This chapter focuses on the interrelationships between weeds and cultivated plants through mycorrhizal networks, as well as on possible herbicide-mediated changes in fungal and plant communities. An overview of the influence of herbicides showing the different modes of action on the formation and functioning of arbuscular mycorrhizal (AM) symbiosis is given. Different issues, such as direct and indirect effects of herbicides on the abundance and diversity of AMF, impact and species-specific responses of AMF to herbicides, and other factors (*i.e.*, mode of action, rate, application method) influencing the effect of herbicides on the abundance and diversity of AMF and AM formation are considered. The possible protective effect of AM symbiosis on crops due to alleviation of herbicide-mediated stress is considered, which could be an important clue for increasing herbicide efficiency. Indeed, in this sense, the use of modern molecular biological tools seems promising.

Keywords: Arbuscular Mycorrhizal Fungi, Crops, Herbicides, Mycorrhizal Symbiosis, Weeds.

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INTRODUCTION

Weeds are a serious problem in agriculture since they cause a potential loss of crop production of up to 34% [1]. Agricultural practices for weed control can significantly reduce such potential crop losses. Modern strategies for crop protection do not involve the complete destruction of weeds through radical methods. Indeed, this could lead to a sharp change in the species composition of agrophytocenoses and the consequent spread of especially harmful species of weeds. Rather than this, the control of weeds to the level of economic threshold of harm [2] is a much more fruitful and ecological strategy.

However, besides the targeted weeds, herbicides can also affect other beneficial components of the soil-plant-environment complex. Among these, arbuscular mycorrhizal fungi (AMF) play a crucial role in soil equilibrium and biodiversity.

Arbuscular mycorrhizas (AM) are the most ancient and widespread symbiosis on Earth, taking place between plant roots and a group of soil-borne fungi (AMF) conforming to the Glomeromycota phylum [3]. This symbiosis appeared over 400 million years ago [4, 5] and is nowadays established between the most terrestrial plants [6]. The fungal symbiont colonizes root epidermal and cortical tissues, forming the intraradical mycelium (IRM), and keeps growing outside the root, forming the extraradical mycelium (ERM), which profusely extends within the soil, thus reaching soil areas that roots can not access by themselves. Therefore, the exploited area for mineral and organic nutrients, as well as water, has greatly increased in mycorrhizal plants. This is of particular importance for slowly diffusing nutrients in the soil, such as P, Cu, Zn, Mn, Fe, and NH_4^+ [7 - 11]. However, besides these beneficial nutritional effects, AM symbiosis reduces losses caused, among others, by drought, salinity, and heavy metal contamination [12 - 16]. AM fungi also play an important role in the functioning of ecosystems, particularly in maintaining soil fertility and improving the growth and survival of plants [17].

Anthropogenic activity can affect the diversity of ecosystem biocoenosis and, in particular, of AM fungi, which may thus become bioindicators of the effects of this activity, including the use of pesticides [18]. In general, when compared to natural ecosystems, agricultural soils have a much lower abundance and diversity of AM fungi [19] and are characterized, for example, by species that are slow-colonizers but more prone to form spores [20]. Consequently, intensive management affects AMF communities, being the most diverse and even AMF communities found in agroecosystems with low or moderate input management [21]. In fact, plant and fungal communities crucially influence each other [22, 23]. AM fungi affect the diversity, productivity, and stability of plant communities, and conversely, changes in plant diversity result in changes in AMF communities [24].

It is important to first consider the effect of AMF on the growth of weeds as well as on cultivated plants without the use of herbicide. This might help to further compare the effects of AMF on weeds when herbicides have been applied and might be useful in understanding AM potential in organic farming.

EFFECTS OF AMF ON WEEDS

As it is well known, not all plant species can form a mycorrhizal symbiosis. Many widespread and troublesome species of weeds belong to the families Brassicaceae, Polygonaceae, Amaranthaceae, Caryophyllaceae, and Chenopodiaceae, many members of which are non-mycotrophic plants [25, 26]. AMF are not host-specific; nonetheless, there is strong evidence for "functional compatibility" among fungal species [27]. Many responses (P uptake, productivity, and resistance to non-mycorrhizal fungal infection) differed depending on the plant-fungus combination, indicating a high degree of fungus- and plant-species specificity.

Based upon fitness effects on plant and fungal partners, symbiotic relationships range from mutualistic, where both partners benefit, commensal, where only one partner benefits, and parasitic, where one partner exploits the other [28]. A fungus can express a mutualism with one plant while simultaneously exploiting a different plant [29]. Egger and Hibbett [28] suggested that the exchange of carbon and nutrients is not the only factor that determines whether mycorrhizal associations are mutualistic or antagonistic. In many cases, by-product benefits or benefits under periods of high plant stress could play more important roles than nutrition itself.

Numerous studies suggest that AMF can affect the nature of weed communities in agroecosystems in a variety of ways, including changes in the relative abundance of mycotrophic weed species "hosts" *vs*. non-mycotrophic "non-host" species [30]. AMF have been shown to induce shifts in host communities by increasing host plant nutrient uptake and growth while suppressing non-mycorrhizal species [31], hence fulfilling the function of "ecosystem engineers".

Competition between plant species affected by different responses to AMF might influence their coexistence. McHaffie and Maherali [32] reported that the AM-positively responding plant, *Plantago lanceolata*, was more strongly limited by intraspecific than the interspecific competition when AMF were present. Conversely, the presence of AMF decreased the strength of intraspecific competition experienced by the AM-negatively responding plant *Bromus inermis*.

CHAPTER 5

Mycorrhiza and its Ecological Significance

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Abstract: Mycorrhizae are important mutualistic associations seen among the majority of terrestrial plants. The plant's roots get infected by a specific group of fungi that enrich the plant in various ways. Though the degree of association varies from one plant to another, researchers and agricultural experts are well aware of the numerous benefits it imparts to the plant. In turn, the fungi gain a nutritional and niche advantage over the other microorganisms in the soil. The fungi involved in mycorrhizal association usually belong to the Ascomycetes or Basidiomycetes groups. Some of these fungi can form simultaneous mycorrhizal associations with multiple plant partners. The specificity and great beneficial aspects of mycorrhizal associations have been adopted to design strategies for increased yield of commercial crops.

Keywords: Ascomycetes, Basidiomycetes, Mutualism, Symbiosis.

INTRODUCTION

Soil is a dynamic ecosystem with an abundance of microbial populations that are constantly interacting with the flora and fauna around it. The soil microflora includes beneficial microorganisms as well as pathogens, which are deleterious to the plants and other organisms around them. In any soil, factors, such as soil moisture, temperature, pH, aeration, the ratio of soil particles, *etc*., influence the microbial load.

The positive interaction between soil communities is ecologically significant as it helps in the effective utilization of available nutrients and the better survival of both communities in the soil. Beneficial interactions include commensalism, synergism, and mutualism (also termed symbiosis). Commensalism is a unilateral relationship where one organism benefits while the other remains unaffected. Synergism and symbiosis are bilateral relationships wherein both the partners benefit, the former being a non-obligatory association, whereas the latter is obligatory.

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Such mutualistic associations greatly alter the soil community dynamics across a wide range of ecosystems. The organisms provide the plants with inorganic nutrients in exchange for plant-derived carbohydrates. While it was originally perceived that bacteria participate in symbiotic relationships for the acquisition of nitrogen and fungi to help with phosphorus uptake, it is now known that fungi are actively involved in the acquisition of almost any limiting nutrient in the soil. One such important soil symbiotic association between fungi and plants, which positively influences the plant's growth, is mycorrhiza. It may be one of the oldest and most evolved associations participated in by terrestrial plants. The term mycorrhiza was coined by botanist Albert Bernhard Frank in 1885. It is formed from two Greek words - myco, signifying fungus, and rhiza, meaning root [1]. In a terrestrial ecosystem, approximately 90% of the plants show mycorrhizal interactions of various types. It is common for a single tree to be associated with several mycorrhizae at a time. 83% of dicots, 79% of monocots and all gymnosperm roots are known to form mycorrhizal associations. Studies on morphological traits and molecular studies of mycorrhiza reveal that ECM fungi are more abundant than AM fungi [2]. Fossil evidence from the Devonian Period (400 million years ago) indicates that early land plants formed endophytic associations with fungi resembling the modern arbuscular mycorrhiza that colonize modern bryophytes [3]. Fossils of spores resembling these fungi have been discovered from rocks of the Ordovician period (460 million years ago), when plants first colonized land (Fig. **1**).

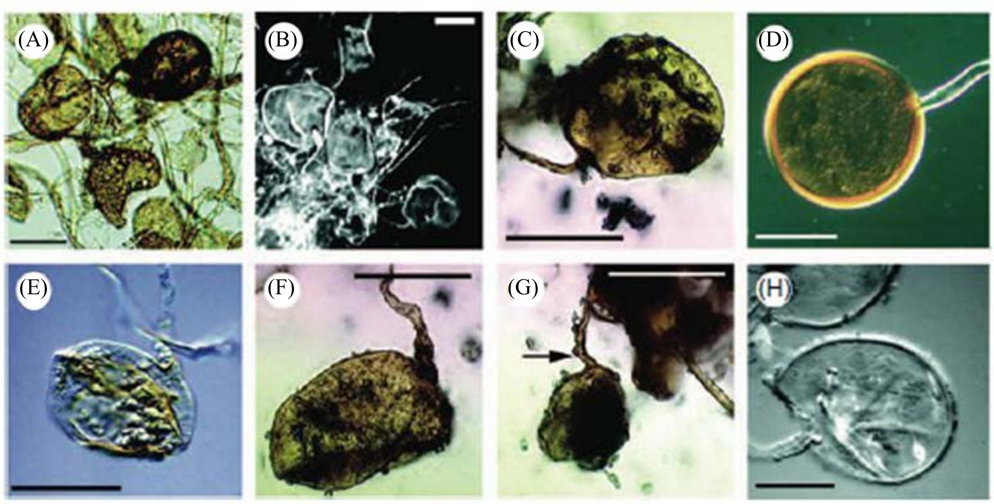

Fig. (1). (**A-C** and **E-G**) Fossil spores from the Ordovician and (**D** and **H**) spores from extinct arbuscular mycorrhizal fungi [4].

TYPES OF MYCORRHIZA

Mycorrhizal classification is based on the degree of interaction of the fungus with the plant and the anatomical features of the root-fungus interface. The two broad groups are ectomycorrhiza and endomycorrhiza. The latter is further divided into subcategories (Table **1**).

Table 1. Comparison between the two most common mycorrhizal associations-ECM and AM.

Ectomycorrhiza (ECM)	**Arbuscularmycorrhiza (AM)**
Intercellular hyphae	Inter and intracellular hyphae
Hypha is septate	Hypha is aseptate
Hartig net and mantle present	Hartig net and mantle absent
Arbuscules absent	Arbuscules present
Fungal genera include *Amanita*, *Boletus*, *Cenococcus*, *Laccaria*, *Inocybe*, *Hebeloma*, *Pisolithus*, *Suillus*, *etc*.	Fungal genera include *Glomus*, *Gigaspora*, *Scutellospora*, *Sclerocystis*, *Acaulospora*, *etc*.

Among the different types known, arbutoid, monotropoid, ericoid, and orchidaceous mycorrhizae can only be found in a few plant species and are, therefore, restricted to certain ecosystems. The distinction between mycorrhizal categories is not necessarily strict as some fungal species can form multiple types of associations (*e.g.*, some fungi form both ectomycorrhizal and ericoid mycorrhizal interactions) [5].

Ectomycorrhiza

In this type of interaction, the fungi do not penetrate the plant cells; instead, they stay associated only with the surface layers of the roots. Ectomycorrhizal (ECM) fungi form a mutually beneficial symbiosis with several species of trees as they do not show a high degree of host specificity. These associations are characterized by two structural features: a compact mantle and a Hartig net. The mantle is a pseudoparenchymatous sheath (resembling plant parenchyma tissue), 20-40 µm in thickness, compactly wound over the root surface (Fig. **2**). The fungal hyphae penetrate the intercellular spaces of the root epidermis and cortical region of the root, leading to an intricately woven Hartig net structure. This serves as the major site of nutrient exchange within the plant root tissue (Fig. **3**).

The mycobiont that forms the ectomycorrhizal association usually belongs to the Basidiomycetes group; occasionally, ascomycetes members are also known to form this type of association. Common examples include fungi of the genera *Boletus*, *Amanita*, *Cenococcus*, *Laccaria*, *Lactarius*, *Inocybe*, *Hebeloma*, *Alpova*,

CHAPTER 6

Mycorrhiza Fungi as a Potential Bioprotectant Against the Plant Pathogens of Chilli

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Abstract: Arbuscular mycorrhiza (AM), a symbiosis between plants and members of the Glomeromycota, an ancient phylum of fungi, boosts the availability of water and nutrients to the host plant, such as phosphate and nitrogen. In exchange, the fungus receives up to 20% of the carbon fixed by the plants. Arbuscules, symbiotic entities found inside plant root cells, are responsible for nutrient delivery. The formation of AM is accompanied by a signalling molecule exchange between the symbionts. Plant roots secrete strigolactones, a new class of plant hormones, which help in host recognition. In India, chilli (*Capsicum annuum* L.) is one of the most important commercial spice crops. After looking over the literature on chilli wilt complex disease, it appears that it causes a major constraint in production. The major diseases affecting chilli production are anthracnose, *Phytophthora* leaf blight, *Fusarium* wilt, bacterial wilt, damping-off, root rot, *etc.* Arbuscular mycorrhiza (AM) is well known for its plant growth-promoting efficiency and providing bioprotection against soil-borne pathogens (bacteria, fungal and parasitic nematodes). Soil-borne plant pathogens are difficult to control by conventional fungicidal methods; therefore, an attempt was made to control the wilt of chilli by eco-friendly management. Increased and efficient use of mycorrhizal fungi may reduce the use of fertilizer.

Keywords: *Capsicum annuum* L., Fungicide, Glomeromycota, Symbiosis.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is a spice vegetable crop cultivated as an annual crop worldwide and originated in Mexico. In the 17th century, the Portuguese introduced chilli to India through Goa [1]. It is preferred for its pungency, spicy taste, and color, which gives a nice flavour to the food. Among five mostly cultivated species of chilli, viz., *Capsicum annuum*, *C. chinense*, *C. baccatum*, *C. pubescens*, and *C. frutescens*, *C. annuum* L. is the most widely cultivated [2]. These are differentiated by their different morphological characteristics, especial-

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ly shape, color, flowers, size of fruits, seeds, and pungency [3]. The major chilli producing states in India are Karnataka, Madhya Pradesh, Andhra Pradesh, Bihar, and Maharashtra [4]. In India, chilli occupies an area of 399 thousand hectares with an annual production of 3737 M tonnes [5]. In Haryana, chilli occupies an area of 18.65 thousand hectares with a production of 130.96 M tonnes [4].

Chilli acts as a natural medicine because it boosts endorphins in the body and increases metabolism. Besides these, it has high values of vitamin A and C. The presence of the alkaloid capsaicin ($C_9H_{14}O_2$) causes pungency in chilli, which is a digestive stimulant and has anti-cancerous properties. Capsaicin is also used in the manufacture of drugs for heart disease, cosmetics, and balms. Some varieties of chilli are famous for their red colour due to the pigment capsanthin and other varieties for their biting pungency, which is due to capsaicin. One hundred grams of the edible part of chilli contains 92.4 percent water, 1.2 g of protein, 0.8 g of fat, 10 g of carbohydrate, 2.6 g of fiber, 11 mg of calcium, and 61 mg of phosphorus [6]. In chilli production, several biotic and abiotic stresses are constraints. The major diseases affecting chilli production are anthracnose, *Phytophthora* leaf blight, *Fusarium* wilt, bacterial wilt, damping-off, root rot, *etc.* Among them, *Fusarium* wilt, caused by *Fusarium oxysporum*, has emerged as a serious problem in recent years. It causes wilt disease in numerous crop plants [7].

Mycorrhizal associations help the host plants to thrive in adverse soil conditions and drought situations by increasing the root surface area and mineral uptake efficiency [8]. Several biocontrol agents are commercially employed these days, such as *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma harzianum*, *T. viride*, mycorrhizal fungi (*Glomus* spp.), *Agrobacterium radiobacter* strain 84 and K1026, *etc.*, against soil-borne pathogens. Among them, the use of mycorrhizal fungi as a biocontrol agent has gained importance in integrated disease management programs [9]. AM fungi are obligate biotrophs, solely dependent on the host plants for their survival. Some bioactive molecules like strigolactones secreted by the roots help fungi to identify their host plants. Strigolactones also stimulate AM fungal growth and its branching. The arbuscular mycorrhizal fungi (AMF) are associated with the majority of land plants, including those in arid areas [10]. Once it is established, it increases mineral nutrition uptake, mainly phosphorus, and enhances plant growth. VAM not only increases the uptake of phosphorus but also helps in the uptake of zinc, copper, sulphur, potassium, and calcium [11]. Mycorrhizal fungi are an important naturally occurring component of the soil ecosystem, as they are associated with the root systems of more than 80% of all terrestrial plant species, including many agronomically important species [12]. Additionally, it protects plants against environmental stress such as soil salinity [13], drought [14] and pathogens such as *Fusarium* wilt [15].

Mycorrhizal fungi, which are the most common symbiotic fungi, spend an important part of their life cycle inside the host. Like pathogenic fungi, most mycorrhizal fungi drain sugars from the host, but they counter this drawback by improving the mineral nutrition of the plant. The common response during these symbiotic associations is a growth increase of the host [16]. Therefore, besides the different responses of the colonized plant at the organismic level, the pathway of the nutrient flux represents an important difference between pathogenic and mycorrhizal associations at the cellular level. It goes from the plant towards the heterotrophic fungus, following a one-way route in pathogenic interaction while there is a two-way exchange in mycorrhizal interaction [17]. In recent years, the use of chemical fertilizers in agriculture has significantly increased throughout the world. It also affects soil fertility, water bodies, and environment. Due to more fertilizers, demand and an increase in the cost of fertilizer, the concept of using mycorrhizal fungi and compost as a biofertilizer and their effect on chilli diseases were studied in this chapter.

MECHANISMS OF MYCORRHIZA

Arbuscular mycorrhizas are the most important symbioses in terrestrial ecosystems, and they enhance the plant's defense against numerous soil-borne pathogenic fungi. Arbuscular mycorrhiza (AM) is well known for its plant growth-promoting efficiency and for providing bioprotection against soil-borne pathogens (bacteria, fungi, and parasitic nematodes) [18]. Several reviews have been written on arbuscular mycorrhiza and biological control of soil-borne fungal diseases of vegetables [19 - 27] and conclude that: (i) mycorrhizal associations can reduce damage caused by soil-borne plant pathogens, (ii) the abilities of the AM symbioses to enhance resistance or tolerance in roots, (iii) protection is not effective for all pathogens, and (iv) protection is modulated by soil and other environmental conditions. There are various mechanisms, and any or all mechanisms may be involved.

Enhanced Nutrition

Excessive land use may have a drastic effect on the overall biodiversity, which in turn may affect the ecosystem function, as shown by various reports [28 - 31]. Arbuscular mycorrhizal fungal colonization is widely believed to stimulate nutrient uptake in plants. Inoculation of arbuscular mycorrhizal fungi can enhance the concentration of various macro-nutrients and micro-nutrients, mainly phosphorus, significantly [32, 33], which increases photosynthate production and hence increases biomass accumulation [34, 35]. Apart from the macronutrients, AMF association has been reported to increase the availability of micronutrients like zinc and copper [28]. Some studies indicate that P-induced changes in root

CHAPTER 7

Role of Mycorrhiza Fungi in Production Agriculture

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Abstract: Mycorrhizae and plants have a well-established symbiotic relationship, and play an important role in better plant growth, disease protection, and improving soil quality. Arbuscular and ectomycorrhizae are the most common of the seven species of mycorrhizae described in the scientific literature (arbuscular, ecto-, ectendo-, arbutoid-, monotropoid-, ericoid-, and orchidaceous mycorrhizae). This chapter presents a summary of current knowledge of mycorrhizal interactions, processes, and potential benefits to society. The molecular basis for genetic exchange between arbuscular mycorrhizal (AM) fungi and host crops, the role of AM fungi in disease protection, in promoting plant growth, in reducing heavy metal load, and in increasing grain production, and their impact on sustainable agriculture are presented in this chapter. The impact of AM-fungal incorporation and beneficial saprophytic mycoflora on the promotion of plant growth and root colonization, the role of AM fungus in restoring indigenous ecosystems, and the impact of the mycorrhizosphere on multitrophic interactions have been summarized. The ways in which the mycorrhizae transform the disturbed ecosystem into productive land are discussed. The importance of restoring mycorrhizal systems in the rhizosphere is emphasized, and their impact on land reclamation and environmental remediation of polluted soils is also discussed. The importance of ectomycorrhiza in forest ecosystems, ectomycorrhizal association in tropical rain forests and their role in maintaining thermal monodominance, are briefly explained.

Keywords: Ecosystem, Ectomycorrhiza, Endomycorrhiza, Mycorrhiza, Plant Defense, Production Agriculture.

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INTRODUCTION

Mycorrhiza is taken from a Greek word mýkēs, which means "fungus", and rhiza, means "root". The term mycorrhiza refers to the role of mold in the rhizosphere of a plant. Mycorrhizae play an important role in plant nutrition, soil biology, and soil chemistry. In mycorrhizal relationships, the fungus encompasses the root tissue of the host plant, either internally intracellularly as in arbuscular mycorrhizal fungi (AMF or AM), or externally as in ectomycorrhizal fungi. The organization sometimes becomes mutualistic. In certain species of animals or certain conditions, mycorrhizae may have an interaction with insects and the plants in which they live.

Mycorrhiza is a symbiotic association between the green plant and the fungus. The plant makes organic molecules like sugar by photosynthesis and provides them to fungi, while the fungus provides plants with water and nutrients, such as phosphorus, extracted from the soil. Mycorrhizas are found in the roots of vascular plants, but mycorrhiza-like associations also occur in bryophytes and there is fossil evidence that early rootless plants formed arbuscular mycorrhizal structures, though some families like Brassicaceae and Chenopodiaceae can not form such an association. The most common are the arbuscular type present in 70% of the plant species, including many vegetable crops such as wheat and rice.

TYPES OF MYCORRHIZA

Mycorrhizas are usually divided into two types: ectomycorrhiza and endomycorrhiza (Fig. **1**). The two types are distinguished by the fact that the hyphae of the ectomycorrhizal fungus do not enter individual cells within the root, while the hyphae of the endomycorrhizal fungus enter the cell wall and invade the cell membrane. Endomycorrhiza includes arbuscular, ericoid, and orchid mycorrhiza, while arbutoid mycorrhiza can be classified as ectendomycorrhiza. Monotropoid mycorrhizas form a special category.

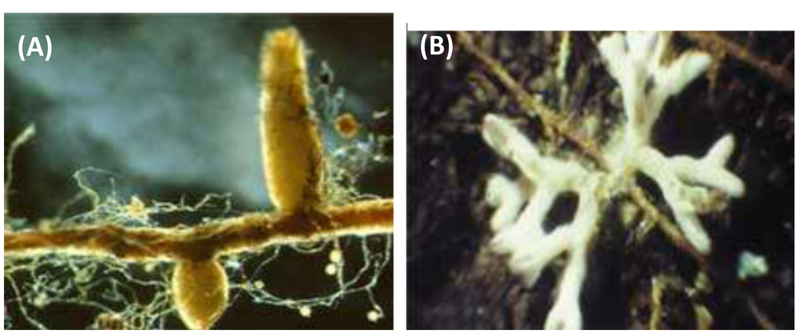

Fig. (1). (**a**) Ectomycorrhiza (**b**) Endomycorrhiza.

Ectomycorrhiza

Ectomycorrhiza, or ECM, is a symbiotic association between the roots of about 10% of the plant family, mainly woody plants including birch, dipterocarp, eucalyptus, oak, pine, and rose families, orchids, and fungi belonging to Basidiomycota, Ascomycota, and Zygomycota. Some ECM fungi, such as *Leccinum* and *Suillus*, are associated with certain plant species, and some fungi, such as *Amanita*, are generalists who create mycorrhiza with a wide variety of plants. Each tree can have 15 or more ECM fungal partners at a time. Thousands of species of ectomycorrhizal fungi exist, which have been transmitted for more than 200 generations. Recent studies have safely estimated the richness of ectomycorrhizal fungi worldwide at about 7750 species, although, based on the known and unknown species in macromycetes, the final estimate of ECM species is approximately between 20,000 and 25,000.

Ectomycorrhizas contain a hyphal sheath or coat, covering the root canal and the Hartig net of hyphae surrounding plant cells within the root cortex. In some cases, hyphae can also enter plant cells, where mycorrhiza is called ectendomycorrhiza. Outside the root, ectomycorrhizal extra metrical mycelium forms a broad network within the soil and leaf litter. Nutrients can be shown to travel between different plants through a fungal network. Carbon has been shown to move from paper birch trees to Douglas-fir trees, thus promoting natural harmony. The ectomycorrhizal fungus *Laccaria bicolor* has been found to attract and kill springtails to obtain nitrogen, some of which can be transmitted to the mycorrhizal plant. In a study by Klironomos and Hart [1], Eastern White Pine fitted *L. bicolor* has been able to extract up to 25% of its nitrogen from springtails. The ectomycorrhizae may contain higher levels of trace elements, such as toxic metals (cadmium and silver) or chlorine, than non-mycorrhizal roots [2].

The first genomic sequence of the symbiotic fungal agent, ectomycorrhizal basidiomycete *L. bicolor*, was published [3]. Multiple gene mutations occur in this fungus, suggesting that symbiosis adaptation continues to replicate genes. Within the genes of a particular gene, those encrypted proteins regulated by symbiosis exhibit a highly regulated expression in ectomycorrhizal root tips that enhances the role of interactions with partners. *L. bicolor* is deficient in enzymes involved in the destruction of plant cell wall components (cellulose, hemicellulose, pectins, and pectates), which prevent symbionts from contaminating host cells during colonization. In contrast, *L. bicolor* has various extended families associated with the hydrolysis of bacterial and microfauna polysaccharides and proteins. This genetic analysis revealed a dual saprotrophic and biotrophic lifestyle of mycorrhizal fungi that allows them to grow within both soil and living plant roots.

CHAPTER 8

Soil Mycorrhiza: Overview, Evolution, Agricultural, and Commercial Applications

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Abstract: Mycorrhiza, meaning fungus root, is a typical example of an endophytic biotrophic and symbiotic relationship rampant in most cultivated and natural ecosystems. Mycorrhizal fungi are fungal species that are closely associated with plant roots, forming a symbiotic relationship resembling legume-rhizobium symbiosis with the plant providing carbohydrates for the fungi and the fungi providing mineral nutrients such as phosphorus and zinc to the plants. Mycorrhizae can enhance the growth of plant roots and even the whole plant system. In addition to nutrient transport, mycorrhizal associations can also impart considerable plant disease resistance against certain plant pathogens. Because of their greater surface area, mycorrhizae can improve plant vigour and soil quality. This chapter deals with the origin and evolution of mycorrhiza using paleontological evidence and phylogenetic analysis of its evolution and its agricultural and commercial applications. Mycorrhizae are important biofertilizers that improve plant nutrition and, thus, productivity by imparting tolerance and resistance to abiotic and biotic stresses and improving soil structure fertility and health and quality.

Keywords: Ectomycorrhiza, Ectendomycorrhiza, Phytoremediation, Soil Health, Stress.

INTRODUCTION

Mycorrhiza-an Overview

Back in 1885, Albert Bernard Frank coined the Greek term "mycorrhiza," which means "fungus roots." They are native to the soil and plant rhizospheres. A plethora of literature is available discussing the importance of mycorrhiza in improving plant vigour by enhancing the uptake of plant nutrients such as phosphorus, zinc, *etc.*, especially from nutrient-deficient soils. Besides, these typ-

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es of mutualistic associations have a role to play in plant protection and soil quality improvement.

In nature, seven types of mycorrhizae, *viz*., arbuscular, ecto, ectendo-, arbutoid, monotropoid, ericoid, and orchidaceous mycorrhizae, are observed. The salient features of these seven classes of mycorrhizae are depicted in Table **1**. Out of these seven types, the most abundant and widely distributed mycorrhizae species are endomycorrhizae, arbuscular mycorrhizae, and ectomycorrhizae [1]. In endomycorrhizal association, the fungi penetrate the cortical cells of roots and form clusters of finely divided hyphae, which ultimately develop into arbuscules (small ellipsoidal structures). While the ectomycorrhizal fungi invade a partial region of the host root without penetrating the cortical cells, they form a thick mantle around the roots.

The most common mycorrhizal association is formed by arbuscular mycorrhizal (AM) fungi, which have a symbiotic relationship with more than 80% of all vascular plants [2]. AM fungi are obligate symbionts belonging to the phylum Glomeromycota [3]. Next to AM fungi, ectomycorrhizal fungi belonging to the phyla Ascomycota and Basidiomycota are widely distributed and associated with 3 percent of vascular plant families (Table **1**) [4].

Table 1. Characteristics of mycorrhizal classes [4].

Mycorrhizal Type	Arbuscular	Ecto	Ectendo	Arbutoid	Monotropoid	Ericoid	Orchidaceous
Fungal taxa	Glomero	Basidio, Asco, and Zygo	Basidio and Asco	Basidio	Basidio	Asco	Basidio
Plant taxa	Bryo, Pterido, Gymno, and Angio	Gymno and Angio	Gymno and Angio	Ericales	Monotropoideae	Ericales and Bryo	Orchidaceae
Intracellular colonization	Present	Absent	Present	Present	Present	Present	Present
Fungal sheath	Absent	Present	Present or Absent	Present or Absent	Present	Absent	Present
Hartig net	Absent	Present	Present	Present	Present	Absent	Absent
Vesicles	Present or Absent	Absent	Absent	Absent	Absent	Absent	Absent
Achlorophylly	Absent	Absent	Absent	Absent	Present	Absent	Present

The characteristics of both ectomycorrhiza and AM fungi are possessed by the fungal species belonging to the class of ectendomycorrhizae, which are found in mutualistic associations with the rhizosphere of many angiosperm and gymnosperm species; fungal symbionts include members of the Basidiomycota, Ascomycota, and Zygomycota. The same fungal species can form either ectomycorrhizal or ectendomycorrhizal associations based on the plant species with which it is associated. Like ectendomycorrhizae, arbutoid mycorrhizae also encompass characteristics of ectomycorrhiza and AM fungi, such as a well-developed mantle, Hartig net, and prolific extra metrical mycelium. In addition, intracellular penetration occurs, and hyphal coils are produced in autotrophic cells. These mycorrhizae are associated with members of the Ericales, namely the *Arbutus* and *Arctostaphylos* species. Monotropoid and orchid mycorrhizae are formed between Basidiomycete fungi and achlorophyllous plant species. Monotropoid mycorrhizae are formed between plants of the Monotropaceae family and a specific subset of fungi in the Russulaceae or Boletaceae family. On the other hand, Orchid mycorrhizae have only been found in association with Basidiomycota [5].

Mycorrhizal application can contribute a lot to increase crop production and maintain sustainable agriculture. However, modern agricultural practices like excess tillage application of chemical fertilizers, following, and nonmycorrhizal crops in crop rotation, *etc.*, can limit the use of mycorrhizae in the field.

ORIGIN AND EVOLUTION OF MYCORRHIZA

The evolution of mycorrhiza is believed to have occurred with that of the first land plants as endomycorrhizal association way back in 450-500 million years, as substantiated by DNA-based phylogenetic analysis and fossil records. The literature revealed that a wide range of endophytic fungal associations was found in most of the terrestrial vascular plants, of which 80-90 percent were viewed as mycorrhizal [6]. Later, as the organic matter content of the soil increased, ectomycorrhiza evolved in and around 150–500 million years. Both endo-and ectomycorrhizal associations progressed in response to environmental changes and slowly became reliant on each other. Hence, it is believed that most existing plants possess a symbiotic association with mycorrhizal fungi. The evolutionary phase of mycorrhiza (Fig. **1**) suggests that it might be a coevolutionary process. In the early phase of the origin of mycorrhizal mutualism, reciprocal genetic exchanges among ancestral plants and free-living fungi might have occurred [7].

Paleontological Evidence of Mycorrhizal Evolution

The ancient fossil record of mycorrhizal evolution was first reported around 400 million years ago in the early Devonian period in the Agalophytes (a Devonian

CHAPTER 9

Soil Inhabitant Bacteria: Journey from Rhizosphere to Eco-Holobiont Approach

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Abstract: The rhizosphere is the most active zone of soil and plays a significant role in soil health management. The rhizosphere concept is more than a century old and has played a pivotal role in understanding the mutual association of microbes and plants over that period. This has opened many interesting facts about wonderful plant-microbe associations. During these years, the concept has evolved from the rhizosphere to the phyllosphere and more recently, to the holosphere/holobiont level. The earlier understanding of how bacteria inhabit plants and, in particular, how bacteria feed plants, has greatly expanded. Recently, it has been observed that plants take bacteria inside their cells and use them as a source of nutrients (rhizophagy). This understanding has completely changed the dimensions of the rhizosphere concept, and we need to think more rationally to understand the bacteria-plant association during the coming years. This chapter covers the wonderful overview of soil-inhabitant bacteria with special emphasis on rhizobacteria in general and plant growth promotion for an enhanced yield of crop plants in particular.

Keywords: Engineering, Rhizosphere Structure, Rhizophagy, *Trichoderma*.

INTRODUCTION

The rhizosphere term was first coined by German agronomist and plant physiologist Lorenz Hiltner in 1904. The term describes the plant-root interface, a word originating partly from the Greek word "rhiza", meaning root [1, 2]. Hiltner described the rhizosphere as an area around a plant root that is inhabited by a unique population of microorganisms influenced by the chemicals released by the plant roots. Simply, the rhizosphere is the soil influenced by roots. Over these years, the rhizosphere definition has expanded to include the three zones, which are defined based on their relative proximity and influence on roots (Fig. **1**). The endorhizosphere includes portions of the cortex and endodermis in which microb-

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es and cations can occupy the "free space" between cells (apoplastic space). The rhizoplane is the medial zone directly adjacent to the root, including the root epidermis and mucilage. The outermost zone is the ectorhizosphere, which extends from the rhizoplane out into the bulk soil.

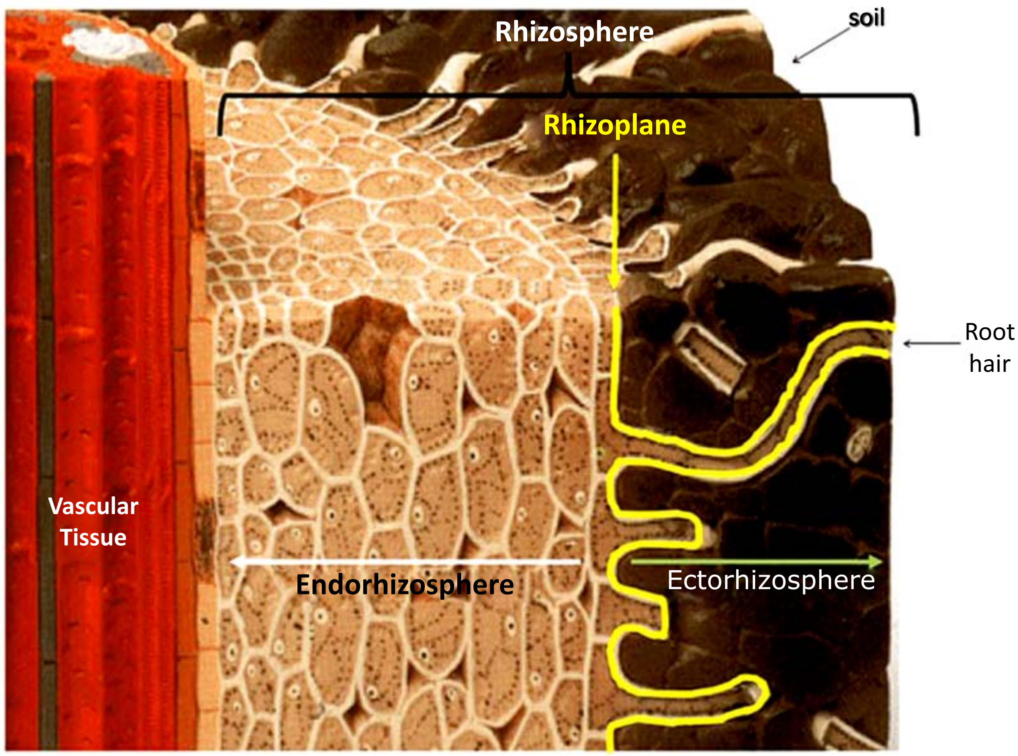

Fig. (1). Schematic presentation of a root section showing the structure of the rhizosphere (permitted the use of the image for educational purposes). (https://www.nature.com/scitable/knowledge/library/the-rhizospher--roots-soil-and-67500617/#).

DIFFERENT COMPONENTS OF RHIZOSPHERE

The rhizosphere zone varies depending on plant species, and its width can be extended from 2 to 80mm away from the root surface [3]. The soil in the rhizosphere supports a typically diverse and densely populated microbial community and is subject to chemical transformations caused by the presence of root exudates and metabolites of microbial degradation. The rhizosphere is characterised by the inherent complexity and diversity of plant root systems, occupying a region of defined size or shape. It also consists of a gradient of chemical, biological, and physical properties, which change radially and longitudinally along the root. Different components of the rhizosphere are described in Table **1** [4].

Table 1. Different components of rhizosphere

Component	Approx Size	Description
Rhizosphere	~cm	Soil influenced by roots
Rhizoplane	1 mm	Root epidermis, mucigel, and adhering soil
Rhizosheath	1 mm	Soil adhered by root hairs and mucilage
P depletion zone	3mm	Concentration gradient of P in soil solution due to uptake
N depletion zone	2mm	Concentration gradient of N in soil solution due to uptake
Accumulation zone	1mm	Calcium from mass flow but not adsorbed
Soil structure modification	1cm	Changes in soil porosity, soil architecture modification
Oxygen depletion	3mm	Oxygen uptake due to root and microbial respiration
CO_2 accumulation	3mm	Respired carbon dioxide from roots and microbes
Exudation zone	2mm	Sugars, mucilage, acids, and allelochemicals released by roots
Microbe	µm–m	Fungal mycelia transcend six orders of magnitude in scale

IMPORTANCE OF RHIZOSPHERE

Proper understanding of the plant rhizosphere has many applications in agriculture, which include:

• PGPR produces certain plant growth hormones (such as auxin, cytokinin, and gibberellin).

• Produces cell lytic enzymes (chitinase, protease, hydrolases, *etc.*).

• Produces secondary metabolites, antibiotics, stress-alleviating compounds (*e.g.*, 1-aminocyclopropane-1- carboxylate deaminase), and chelating agents (siderophores)

• Produces some signalling compounds (*e.g.*, N-acylhomoserine lactones) to interact with the beneficial or pathogenic counterparts in the rhizosphere.

• Use of plant growth-promoting organisms and the suppression of plant diseases.

• Rhizosphere organisms can be used to enhance the formation of stable soil aggregates and as bioremediation agents for contaminated soils.

• The rhizosphere zone has increased nutrients, biotic activity, and interactions that help in manipulation to improve plant productivity and environmental quality.

CHAPTER 10

Cyanobacterium: Uses as a Biocontrol Agent, Biofertilizer, and Plant Growth Promoter in Agriculture and Environmental Sustainability

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Abstract: Cyanobacteria continue to produce various biologically active compounds of antibacterial, antifungal, antifungal, and antiviral potential. These bioactive compounds also belong to the groups of polyketides, amides, alkaloids, fatty acids, indoles, and lipopeptides. In addition, these cyanobacteria often produce a broad spectrum of anti-algal compounds that attempt to inhibit the growth of pathogens by inhibiting their metabolic and physiological activities. We all know that cyanobacteria were among the first microorganisms to live on Earth. Long ago, about billions of years ago, they played a major role in shaping the Earth into the planet we live on today, and they play an important role in a variety of functions in addition to our daily lives. Despite the small genome of cyanobacteria, marine cyanobacteria are also prolific secondary metabolite producers, along with being an essential source of atmospheric oxygen. With the ever-increasing human population and higher post-production waste emissions and increased use of fossil fuels based on food requirements, its concentration in the atmosphere is expected to increase steadily. Since most of the attention related to metabolite production has historically been focused on their freshwater counterparts, marine cyanobacteria present a relatively untapped resource in terms of evolutionary diversity and industrial potential. They are also producers of several complex secondary metabolites with potential applications in human health, biofuels, and bioengineering.

Keywords: Cyanobacteria, Antibacterial, Antifungal, Anthropogenic, Usar land.

INTRODUCTION

As of now, the current world population of about 7.2 billion is expected to continue to grow to surpass 9.6 billion by the end of 2050. In this situation, the challenging target would require an increase in the annual production of cereals by about 50%, *i.e.*, 2.1 billion tons to 3 billion tons per year, to provide food for all due to the ever-decreasing agricultural holdings, which seems almost impossi-

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ble. This difficult goal, which is impossible, puts immense pressure on the agriculture sector to achieve food security for all through food production. But such a big goal in food production can be achieved either by using more and more land for farming or it can be possible only by increasing the productivity of available arable land. In light of limited agricultural land holdings and an increasing population, the first option for food security has become a distant dream. If soil fertility is increased with the help of better eco-friendly management tools, then the alternative of increasing agricultural productivity can be fulfilled. Current agricultural practices and the use of synthetic fertilizers and pesticides rely heavily on intensive tillage as well as excessive irrigation, which have undoubtedly helped many developing countries almost meet their people's food needs; this has led to increased environmental and health problems, including a decline in soil fertility, a polluted environment with overuse of land and water resources, and increased costs of agricultural production.

A major question that arises in the face of current agriculture is whether agricultural production can be increased continuously to meet the population's present and future essential food needs without reducing the quality of the environment [1]. Sustainable farming practices can meet our growing need for quality food as well as our environmental quality. At present, under sustainable agriculture, eco-friendly, low-cost farming can be included with the help of native microorganisms [2]. Now we also have to emphasize that all of us, as well as farmers, must work with natural processes to conserve resources like soil and water, along with the cost of agricultural production and our environment. Various waste generation, which adversely affects the quality, should be reduced. By adopting such sustainable agriculture management practices, the global agroecosystem can be made more resilient and self-regulating, and productivity and profitability can also be maintained.

In research for a long time, various microbes have been known to make valuable contributions to soil fertility and the production of sustainable green energy. During the research of the last decades, microbial processes of green energy production have gained special recognition as a sustainable tool for the production of several bio-fuels, namely methane (CH_4), ethanol, H_2, butanol, syngas, *etc.* The current scenario shows a significant increase in the production of cyanobacterial biomass for many biofuels, various food supplements (superfoods) and biofertilizers for safe agriculture [3]. Ruffing [4] has classified beneficial as well as harmless bio-agents based on their role in regulating plant productivity in his research. These two diverse groups of microorganisms co-exist in nature, and it has often been found that the predominance of one at a time depends largely on environmental conditions. For many years, soil microbiologists and microbiologists have been studying the effects of beneficial or efficient soil microorganisms for sustainable agriculture that not only increase soil fertility but also increase crop growth and yield and contribute to the improvement of the quality of the environment. Today, the adoption of sustainable farming practices demonstrates the important role of all these useful small microorganisms in achieving food security without causing many environmental problems. It has often been found that the trend of using different types of bio-inoculants containing beneficial soil microbes over synthetic fertilizers, insecticides and insecticides is proving to be a beneficial step for increasing crop productivity. Cyanobacteria as beneficial microbes in agriculture have the potential to play a potential role in increasing agricultural productivity and reducing GHG emissions. As a result of recent research, it has been proposed that these cyanobacteria may be important bioagents in many ways in the re-ecological restoration of many types of degraded lands. These cyanobacteria have always been a group of photosynthetic organisms that can easily survive on the minimal requirements of light, carbon dioxide (CO_2), and water in many ways.

Cyanobacteria are a form of phototrophic that occurs naturally in many agroecosystems, such as rice fields and under rough conditions from Antarctica to the Arctic poles. They often meet their nitrogen requirements through nitrogen (N_2)-fixation and also produce some bioactive compounds that promote crop growth and protect them from pathogens, among other things. They also improve the nutrient status of the soil [5]. Recent research has also found that cyanobacteria are also very useful for wastewater treatment and can reduce the effects of a variety of toxic compounds and even pesticides. A conceptual model regarding the role of cyanobacteria in sustainable agriculture and environmental management has also been proposed in several countries. Following a research review, cyanobacteria shed light on their effective role in bioenergy production, ecological restoration, agriculture, and environmental sustainability.

CYANOBACTERIA IN SUSTAINABLE MANAGEMENT

Cyanobacteria, a beneficial pathogen, have always been a useful alternative to other management practices. Cyanobacteria have the ability to fix atmospheric N_2 as well as decompose many types of organic wastes and residues, detoxify heavy metals present in the soil, many types of pesticides and other xenobiotics, and catalyze the nutrient cycle. It is also capable of killing pathogenic microorganisms in soil and water. It inhibits bioactive growth and is capable of producing some bioactive compounds as well. Cyanobacteria play an important role in the production of compounds such as vitamins, hormones, and enzymes that contribute to plant growth.

CHAPTER 11

Industrial Aspects of Soil Microbes

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Abstract: The multifaceted potential of soil microorganisms is being exploited in various fields like agriculture, food and cosmetic industries, for the sustainability of the environment and in the industrial production of useful compounds. On the one hand, these microorganisms play an essential role in the nutrient cycling of minerals like phosphorus and nitrogen that are crucial for their survival and sustenance, along with making the soil fertile by releasing important growth-promoting hormones like ethylene, auxin, and cytokinin. On the other hand, the potential of soil actinomycetes like *Dactylosporangium*, *Ampullariella*, *Actinoplanes*, *Actinomadura*, and *Actinosynnema* is being explored extensively for the industrial production of new life-saving antibiotics. Many of the enzyme producing species like *Streptomyces ruber*, *S. lividan*, and *S. rutgersensis* are used in supplements for detergents, textiles, animal additives, paper, and pulp. *Xanthomonas* produces xanthan gum, which is used to thicken and stabilize foods and cosmetics. Screening desired microorganisms and manipulating them to obtain maximum production is a crucial step in industrial production. Hence, it can be concluded that soil microorganisms are important for diverse metabolite production useful in agriculture and industry as well as having the capability to transform recalcitrant compounds to reduce environmental pollution.

Keywords: Soil Microbes, *Xanthomonas*, Industrial Production, *Actinomycetes*, Single Cell Protein.

INTRODUCTION

Microorganisms have existed for a very long time on earth. Our planet is 4.6 billion years old, approximately. As per literature, the first microorganisms were about 3.86 billion years ago in the form of stromatolites, layered sedimentary formations that are created by photosynthetic cyanobacteria. We are all surrounded by microorganisms and can not imagine life without them. Some of them are harmful, but many of them are useful for human and industrial purposes. They are crucial for the production of a variety of metabolites that help in agricul-

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ture as well as the transformation of chemicals that help to reduce environmental pollution. The development of microorganisms and manipulation in such a way that maximum production can be obtained is a crucial step in industrial production [1].

Soil microbes are important for the development of a healthy soil structure. Abundant soil organic carbon improves soil fertility and water-retaining capacity. Soil is an ecosystem capable of producing the resources necessary for the development of living organisms. Soil microorganisms participate in a variety of biogeochemical processes, including organic matter mineralization, proteins and nucleic acids synthesis, nutrient cycling, phosphorus forms transformation, organic matter decomposition, biodegradation of impurities and soil structure maintenance. Soil microorganisms may provide a significant means of reducing atmospheric greenhouse gases and help to limit the impact of greenhouse gas-induced climate change [1].

Nutrient cycling is critical to Earth's survival; otherwise, essential nutrients would be rapidly taken up by organisms and locked in a form that others could not use. The reactions involved in elemental cycling are often chemical in nature, but biochemical reactions, those facilitated by organisms, also play an important part in the cycling of elements (Fig. **1**). Soil microbes are generally bacteria, actinomycetes, fungi, protozoans, and nematodes.

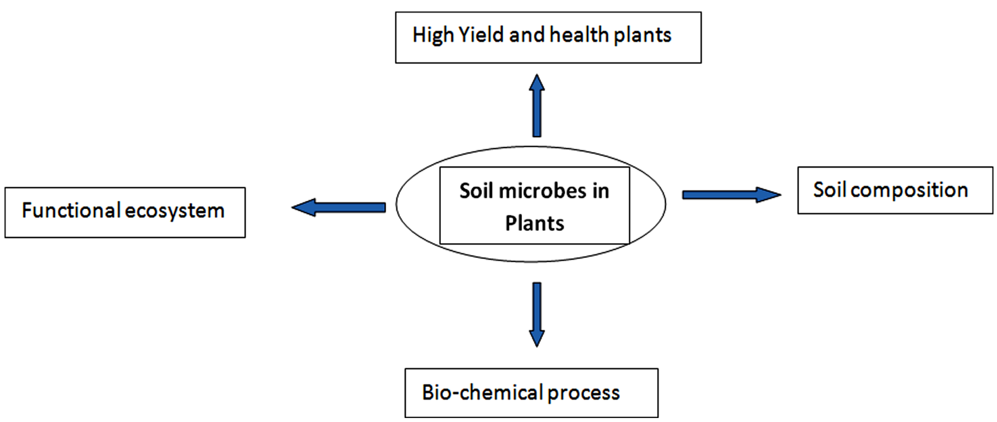

Fig. (1). Functions of soil microorganisms [2].

BACTERIA

Bacteria make up the most metabolically diverse group of living organisms. Although some are parasitic on animals and plants, the majority of bacteria are free-living, having either a neutral or beneficial relationship with humans and other animals and plants. Their metabolic versatility is incredible, while most are heterotrophs, using either light or chemical energy. One of their most remarkable characteristics is their ability to multiply rapidly. Nitrogen-fixing bacteria form a symbiotic relationship with legumes, which convert atmospheric nitrogen into ammonia [3]. Various bacteria produce diverse end products during anaerobic sugar fermentation reactions. For thousands of years, brewer's yeast, *Saccharomyces cerevisiae*, has been used in the production of alcohol and is now being used for fuel production due to lower distillation expenses and a high yield of ethanol. Bacteria like *Acetobacter* are used in the production of vinegar by oxidizing alcohol to acetic acid. A few industrially important compounds produced by bacteria and fungi along with their applications are listed in Table **1**.

Table 1. List of industrially important compounds produced by bacteria and fungi.

Products	Bacterium	Application
Proteases	*Bacillus* sp., *Serratia* sp., *Xanthomonas* sp., and *Candida* sp.	Food and beverages, textile, leather, paper, pharmaceuticals, cosmetics, and detergent industry
Cellulases	*Clostridium thermocellum*	Agriculture waste processing and paper industry
Amylases	*Bacillus* sp., *Aeromonas* sp., and *Alteromonas* sp.	Beverages, starch industry, paper, pharmaceutical, and food industry
Lipase	*Pseudomonas* sp.	Food, textile, leather, paper, pharmaceuticals, cosmetics, and detergent industry
Glucose isomerase	*Bacillus coagulans*	Sweetener in the food industry
Beta-galactosidase	*Thermus aquaticus*	In the hydrolysis of lactose in milk whey to glucose and galactose
Cellobiose- hydrolase	*Thermus maritime*	Cellulose degradation
Taq polymerase	*Thermus aquaticus*	PCR technology
Glucose oxidase	*Aspergillus niger* and *Penicillium glaucum*	Glucose testing strips, food, pharmaceuticals, and biotechnology industry
Vinegar	*Acetobacter* spp.	Food and beverages, cleaning industry, agriculture, and healthcare industry
Monosodium glutamate	*Micrococcus* spp.	Food processing industry
Dextran	*Leuconostoc mesenteroides*	Cosmetic and pharmaceutical industry

Biocontrol Agent

A *Bacillus thuringiensis* formulation has been marketed for years as a biocontrol agent for caterpillars. Liquid formulations of Serenade, a biopesticide containing the *Bacillus subtilis* strain, can be sprayed over plants and into the soil to exhibit antifungal and antibacterial properties. It has also been reported to promote plant growth and has found acceptance in both conventional and organic agriculture [4].

CHAPTER 12

Microbial Biotransformation in Steroids Production

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Abstract: Biocatalysis is also widely applicable for the enhancement of bioprocess productivity, selectivity of target reactions, and production of valuable chemicals, pharmaceutical ingredients, precursors, and key intermediates. The vast majority of such enzymes are of microbial origin and include dehydrogenases, oxygenases, hydrolases, transferases, and lyases. These enzymes may introduce minor molecule changes, such as the insertion of a hydroxyl or keto function or the saturation or desaturation of a complex cyclic structure. Microorganisms and cell suspension cultures of the plant are applied in biotransformations of steroidal drugs to generate high regio-and stereo-selective products. Studies of steroid modifications catalyzed by microbial or plant cell cultures represent a well-established research approach and methodology in biotechnology. Bioconversion can occur at a position of the steroid molecule that is rarely accessible to chemical agents; the molecule can function stereospecifically, and several reactions can be completed in a single biotechnological step.

Keywords: Hydroxylation, Microbial Transformation, Plant Cell Cultures, Steroidal Drugs, Whole-Cell Biocatalysis.

INTRODUCTION

A steroid is a biologically active organic compound with four rings arranged in a specific molecular configuration. Steroids have two principal biological functions: as important components of cell membranes that alter membrane fluidity; and as signalling molecules. Hundreds of steroids are found in plants, animals, and fungi. All steroids are manufactured in cells from the sterols lanosterol (opisthokonts) or cycloartenol (plants). Lanosterol and cycloartenol are derived from the cyclization of the triterpene squalene.

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Hormones can be of three types based on the chemical structure: (i) proteins (*e.g.*, FSH and LH), (ii) peptides (*e.g.*, GnRH and ACTH), and (iii) steroids (*e.g.*, progesterone and estradiol). The amino acids are the basic units of proteins and peptides, whereas the gonane structure is present in steroids. Compared to proteins, steroids normally consist of small molecules and are soluble only in organic solvents. The molecular weight and solubility of peptides fall somewhere between these two categories of hormones.

CHEMISTRY OF STEROIDS

The steroid core structure is typically composed of seventeen carbon atoms, bonded in four "fused" rings: three six-member cyclohexane rings (rings A, B and C in the first illustration) and one five-member cyclopentane ring (the D ring) (Fig. **1**). Steroids vary by the functional groups attached to this four-ring core and by the oxidation state of the rings. Sterols are forms of steroids with a hydroxy group at position three and a skeleton derived from cholestane. Steroids can also be more radically modified, such as by changes to the ring structure, for example, cutting one of the rings. Cutting Ring B produces secosteroids, one of which is vitamin D_3. Lipid cholesterol, sex hormones (estradiol and testosterone), and their use in anti-inflammatory drugs are some examples.

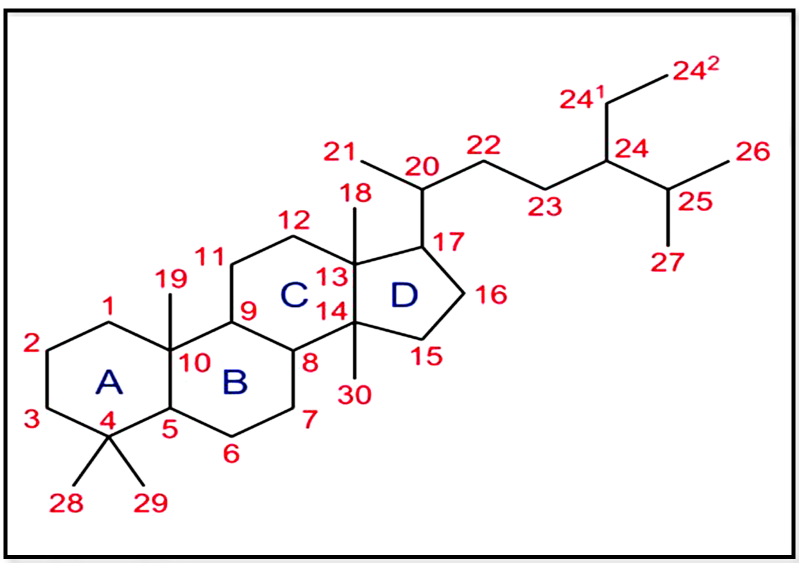

Fig. (1). Structure of cholestane, a steroid with 27 carbon atoms. Its core ring system (ABCD) is composed of 17 carbon atoms.

Steroids are classified based on the number of carbons (Fig. **2**). Biotransformation (regiospecific and stereospecific bioconversion) is a biological process whereby an organic compound is modified into a reversible product. These involve simple, chemically defined reactions catalyzed by enzymes in the cell.

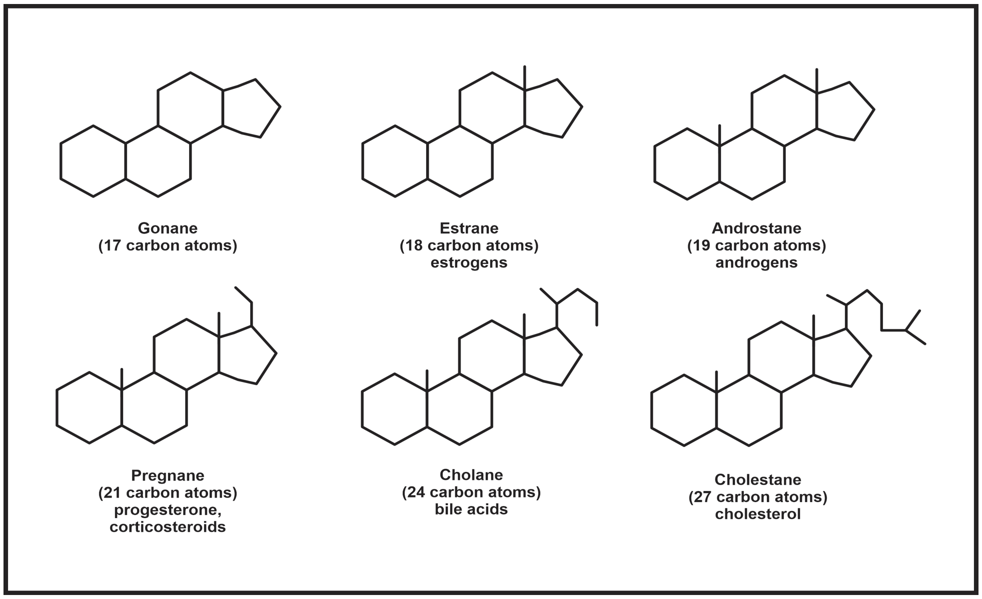

Fig. (2). Steroids are classified by the number of carbons in their molecules.

If microorganisms carry out the transformation of organic compound compounds, then the process is called microbial transformation. Naturally occurring steroids possess remarkable hormonal properties which are of therapeutic importance to human beings, such as hormones of the adrenal cortex (cortisone, cortisol, corticosterone), the progestational hormone (progesterone), the androgens or male sex hormones (testosterone, dihydrotestosterone) and the estrogens of female sex hormones (estradiol, estrone). Depending on the functional group of the molecules of each parent steroid, the compounds differ in their characteristics. Generally, ketone groups, hydroxyl groups, and double bonds are common functional groups (Fig. **3**). The carboxyl and aldehyde groups are other functional groups present in the molecules of bile acids and aldosterone, respectively.

Microorganisms possess the capability to modify a wide range of organic compounds enzymatically. Bio-transformations (bi-conversions or microbial transformations) broadly refer to the processes in which microorganisms convert organic compounds into structurally related products. In other words, biotransformation deals with a limited number of enzymatic reactions (one or a few). This is in contrast to fermentation, which involves a large number of reactions and is often complex.

CHAPTER 13

Endophytes as an Alternative Source for Anticancer Agents

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Abstract: The world faces new challenges every decade in the form of calamities, pandemics, and deadly diseases. The increase in the population and limited resources has led the human race towards many ailments that are incurable, but the potency of the human brain and in collusion with natural resources can reveal the remedy to many diseases. Cancer is one of the major reasons for mortality at present, which is a global challenge. The search for new anticancer drugs is a necessity of the present day. Researchers are urged to explore alternative and new potent sources of anticancer drugs. Natural sources include plant products or some plant-derived bioactive compounds. Endophytes manifest as an acceptable source of bioactive compounds of medicinal value. Endophytes are microorganisms present asymptomatically inside the plant parts. These are known to produce several metabolites with antifungal, antiviral, antioxidant, and anticancerous activity. Some major metabolites include taxol, alkaloids, camptothecin, chromones, *etc.* These produced metabolites can also be manipulated for the production of novel chemotherapeutic agents. The incessant need for these anticancer drugs has escalated the search for novel natural compounds. The present chapter attempts to summarize different endophytic metabolites that serve as an alternative source for an ailment of the deadly cancer disease.

Keywords: Anticancer Agents, Camptothecin, Chromones, Endophytes, Taxol.

INTRODUCTION

Humans have always searched for new natural products throughout the decades for various purposes. Most natural products are plant-based or microorganism-based. Many of the natural products from plants and microorganisms are explored, but many more are in the queue and are being discovered day by day. Microbial natural products are untapped resources of unique bioactive compounds formed during evolution in exchange for response to the environment and plant habitats [1].

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Endophytes are microorganisms that are present inside the host plant body (the interstitial spaces of healthy plants and exhibit complex interactions with their host) without showing any external symptoms, *i.e.*, they may have a beneficial role but are not reported to cause any harm to the host plant. Naturally, every plant possesses endophytes. Exploration is needed to seek out novel strains of endophytes. Endophytes are valued (mainly endophytic fungi) for their ability to produce many useful bioactive metabolites with medicinal properties, including antiviral, antibacterial, antifungal, and anticancer activity [2], which can be further explored in the discovery of novel drugs. Most plants act as hosts for endophytes, and one million endophytes are estimated to be present [3].

The world facing an exponential rise in health issues due to cancer is one such concern. According to a UN report, 7.4 million deaths (13% of all deaths) in 2004 were just because of cancer. Among these, 70% of deaths occurred in low-and middle-income countries. By 2030, the estimated death toll will be 11.5 million. Cancer cells are characterized by abnormal replication potential, apoptotic resistance, and invasive ability. Some anticancer drugs reveal toxicity to escalating normal cells and possess inimical effects; therefore, there is a need for bioactive compounds from natural products [4]. Approximately, 57% of compounds from natural sources, *i.e.*, plant-based like taxol, vincristine, etoposide, irinotecan, topotecan, and vinblastine, *etc.*, are in clinical use for the treatment of several human cancers. Natural products used in cancer treatment are not always ecologically and economically feasible, as to cure one patient of cancer, there is a requirement of six yew trees of 100-years old. Only 0.01% to 0.03% taxol content can be obtained from the dry weight of the phloem of the single yew tree [5]. The estimated cost of the development of a new drug is around $2.6 billion, and annual expenditure increased to $150 billion in 2020 [6].

Cancer cells show a high rate of mutation, which compelled the scientific community to search for novel anti-cancer compounds. Endophytes need to be explored for the isolation of bioactive secondary metabolites that provide molecules with properties and importance, such as xanthones, tetralones, terpenoids, quinones, steroids, chinones, benzopyranones, alkaloids, and phenolic acid having a wide range of pharmaceutical applications. This has led to a shift in current research towards finding an efficient reliable, prudent, environmentally secure and alternative method for the isolation of bioactive compounds from microorganisms. Endophytes can be one of the potential sources of anticancer agent. Endophytes serve as a good option as they are not so difficult to isolate and culture and could be used for the extraction of plant-based bioactive metabolites. Secondary metabolites that are isolated from endophytic fungi have been recognized as a potential source of anticancer compounds [7 - 10]. Since the discovery of fungal-derived paclitaxel, various compounds, including camptothecin and several analogues [11 - 13], vincristine and podophyllotoxin [14, 15] with anticancer activity have been isolated [16]. L-asparaginase is an important anticancer molecule, which is often sourced from the genus *Colletotrichum*, followed by species of *Fusarium*, *Penicillium*, and *Phoma* [17]. Camptothecin [11], an anticancer drug that was first isolated from the bark of *Camptotheca acuminata.*

Several reports have described other camptothecin (or analogues) producing endophytes. Endophytic fungi belonging to the genera A*lternaria*, *Aspergillus*, *Ectostroma*, *Botryodiplodia*, *Botrytis*, *Cladosporium*, *Fusarium*, *Metarhizium*, *Monochaetia*, *Ozonium*, *Papulaspora*, *Pestalotia*, *Pestalotiopsis*, *Phyllosticta*, *Pithomyces*, *Mucor*, *Periconia*, and *Taxomyces* are the natural sources of paclitaxel and its analogues [18]. Microbial fermentation is a reasonable and alternative method to extract taxol from the endophyte *Taxomyces andreanae* [19]. Taxol has also been found in several different genera of fungal endophytes, either associated or not in use, such as *Taxodium distichum* [20], *Wollemia nobilis* [21], *Phyllosticta spinarum* [22], *Bartaliniaro billardoides* [23], *Pestalotiopsis terminaliae* [17], and *Botryodiplodia theobromae* [24].

Moreover, the deciphering and manipulation of novel metabolic pathways for the mass production of anticancer compounds will be a critical step for the development of cost-effective cancer treatment. Bioactive compounds isolated from endophytes show cytotoxic effects against specific cancer cells. There is a need in natural product research to adopt a valuable approach so that from unexplored areas of the endophytic domain, we might get new and interesting secondary metabolites that have several biological applications. From the literature, it is evident that bio-prospecting of fungal endophytes always remains a priority during a search for anti-cancerous natural molecules.

The microorganisms living inside asymptomatically during the life cycle of the plant are known as endophytes. The endophytes are different from the epiphytes that are found on the surface of the plant. Fungi, bacteria, Protista and Archaea are the microorganisms contributing to the endophytic population. These microorganisms have a mutualistic relationship with the host plant despite the different types of stress available to the plant. The endophytes are reported to promote plant growth, help with disease resistance, stress tolerance, secondary metabolite production, and bioremediation [25]. Endophytes also produce important natural products for the host plants, such as alkaloids, terpenoids, steroids, peptides, *etc.* The discovery of new natural products and compounds from these plant-based microorganisms will help open new vistas towards the cure of fatal diseases. The quest for new potent drugs has always persisted throughout human history.

CHAPTER 14

Carotenoids in Microorganisms and Their Applications

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Abstract: Naturally occurring carotenoids' demand is increasing because of their need in the pharmaceuticals, food, cosmetics, flavor, and animal feed industries. Extraction and synthesis of carotenoids are expensive and technically challenging. To fulfil the ever-increasing demand for the production of carotenoids, microbial production of carotenoids seems to be an attractive alternative to current extraction from natural sources. For carotenoid overproduction in microorganisms, metabolic engineering as well as synthetic biology strategies, have been extensively used to reconstruct and optimize pathways of carotenoid production. Modified and advanced strategies such as the novel and specific enzymes, protein engineering, target gene screening, and regulation tools should be used to improve carotenoid production. The applications of carotenoids, biosynthetic pathways of metabolic engineering of microbial carotenoid production, molecular breeding of carotenoids, and prospects of carotenoids are discussed in the present review.

Keywords: Carotenoids, Microbial Production, Metabolic Engineering, Synthetic Biology.

INTRODUCTION

Naturally, derived pigments are predominantly represented by carotenoids, flavonoids (anthocyanins), and some tetrapyrroles (chlorophylls and phycobiliproteins). Carotenoids are one of the most commonly available natural products. Carotenoids produce a subfamily of terpenoids that normally contain 40

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carbon atoms, have 8 isoprene molecules, and contain orange, yellow, and red hues that are attributable to an extensively conjugated polyene chain. The demand for carotenoids is increasing due to their extensive uses in the flavors, pharmaceuticals, cosmetics, foods, and feed industries. However, the extraction and synthesis of carotenoid compounds can be expensive and technically challenging [1]. Several species of algae, fungi, and bacteria have been exploited commercially for the production of pigments [2].

The typical chemical structure of carotenoids is responsible for their physiological function as provitamin A nutrients and antioxidants, as well as their potential to protect against UV radiation. Besides, carotenoids have a very important role in apoptosis in mammalian cells and in gene regulation, as reported by many research workers [3], which are metabolic processes needed for maintaining the animal's health. Carotenoids play an important role in fungi, such as potent photoprotective and stress-protecting agents [4]. An ideal pigment producing microorganism should be capable of using a wide range of C and N sources, must be tolerant to pH, temperature, and minerals, and must give reasonable colour yield. The non-toxic and nonpathogenic nature, coupled with easy separation from cell biomass, are also preferred qualities. Microbial, specifically bacterial carotenoid production, provides a better alternative to the increasing negative environmental impacts, direct extraction from plants and high cost of chemical synthesis [5]. Bacterial pigments have many advantages over artificial and inorganic colours. The bacterial growth rate is higher than that of other microbes, such as algae and fungi. The other enduring strength of bacteria is their relatively large and easily manipulated strands of genes. Besides, pigment production from bacteria is independent of weather conditions, which produce different colour shades and grow on cheap substrates [6].

According to McWilliams [7], the global market for carotenoids is reported to be worth $ 1.5 billion in 2018 and will reach an estimated value of $2.0 billion in 2022. However, carotenoid demand in the market is increasing rapidly due to insufficient production, and their supply is limited [5, 8, 9]. The sources of extraction of mostly carotenoids are natural sources, so yields are low because of complicated multi-step processes. The concentration of carotenoids is low in natural raw materials, which can be affected by deteriorating environmental conditions [10].

Metabolic engineering and synthetic biology technologies have been appreciated and applied to the reconstruction and optimization pathways for the overproduction of carotenoids in microorganisms [11]. Bacterial pigment production can be increased in geometric proportions through genetic engineering, compared to the scaling-up methods of chemists. Microbes also have an upper hand in versatility and productivity over higher forms of life in the industrial-scale production of natural pigments and dyes [2].

Over the past few years, a considerable number of plant and microbial carotenoid biosynthesis genes have been cloned. Through functional heterologous expression, it has become possible for most of these genes to engineer carotenoid biosynthesis in non-carotenogenic yeasts and *Escherichia coli*. Nowadays, novel and rare high-value carotenoids are being produced through gene combinations and molecular breeding pathways.

Metabolic engineering is, in a narrow sense, defined as a purposeful modification of metabolic networks in living cells to produce desirable chemicals with superior yield and productivity using recombinant DNA techniques [11 - 13]. Its main concerns are investigations undertaken to produce chemicals of commercial interest efficiently and abundantly using appropriate microbes [14]. It has traditionally been postulated that the production hosts are microbes that naturally synthesize the desired chemicals. However, microbes that can produce precursors of the desired chemicals with superior yield and productivity are also considered hosts [15]. This notion significantly extends the scope of utilizable microbes as productive hosts. The main objective of metabolic engineering of microbial carotenoid production is to engineer appropriate microbes that can produce large amounts of desirable carotenoids efficiently in their cells, regardless of their natural ability to synthesize carotenoids.

As the inventory of cloned genes and entire genomes grows exponentially, we are acquiring the ability to harness and manipulate biosynthetic pathways in native and heterologous hosts. Because plants, algae, and microbes synthesize hundreds of different complex chemical carotenoid structures [16], several carotenoid biosynthetic pathways have been elucidated on a molecular level. Metabolic engineering of microbes can provide a means for the economical production of carotenoids that are otherwise inaccessible.

Carotenoids are important natural pigments displaying yellow, orange, and red colours and are produced by microorganisms like algae, fungi, bacteria, and green plants. Along with very few bacterial carotenoids with 30, 45, or 50 carbon atoms, C40 carotenoids constitute the majority of the more than 600 known structures. But most of these carotenoids are biosynthetic intermediates. These intermediates accumulate in traces, making it difficult to extract a sufficient amount of material for their purification and other applications [17].

A potential mercantile interests in the mass production of sustainable industrial carotenoids leading to research breakthroughs that rapidly increase carotenoid tit-

CHAPTER 15

Interaction of Plant-parasitic Nematode and Filamentous Fungi: an Insight Story of Mechanism Involved and Tool for Sustainable Agriculture

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Abstract: Phytoparasitic nematodes are highly dangerous to the global agricultural production of a variety of crops. Chemical nematode overuse necessitates the creation of new nematode control strategies. Filamentous fungi could be a feasible biocontrol alternative in this case. *Trichoderma*, mycorrhizae, and endophytic fungi are the most common filamentous fungi studied and used as biological control agents (BCAs) against nematodes as resistance inducers. Several pathways have been linked to the biocontrol effect of fungi on plant-parasitic nematodes. Increased plant tolerance, direct competition for nutrients and space, induced systemic resistance (ISR), and altered rhizosphere interactions are all possible pathways. Several mechanisms, as well as a detailed discussion of their plausibility in the biocontrol of plant-parasitic nematodes, in particular, have been postulated. Mycorrhizal fungi are not yet widely utilized in conventional agriculture, but recent data is assisting in the development of a better understanding of the mechanisms of action. This will eventually lead to mycorrhizal fungi being used in the field to combat plant-parasitic nematodes.

Keywords: Biocontrol, Fungi, Plant-parasitic Nematodes, Plant Systemic Resistance.

INTRODUCTION

Plant-parasitic nematodes (PPN) are pests that can infect a wide range of important crops. Some species, like *Ditylenchus* spp., can be pests aboveground despite living primarily in the soil. PPN live in a variety of ways, but they always eat with a stylet, which is a hollow, retractable, needlelike mouth spear. They are classified as ectoparasitic, partly endoparasitic, or endoparasitic [2], depending on

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their feeding technique [1]. Ectoparasitic nematodes spend their entire life cycle outside of the host, with only the insertion of a long and rigid feeding stylet making physical contact. Semiendoparasitic nematodes feed by invading roots and leaving the posterior part in the soil. Endoparasitic nematodes enter the root system altogether and feed on underlying tissues. The root-knot nematode (RKN) and cyst nematodes (CNs) are among the most damaging nematodes [3]. PPN-related yield losses are expected to increase in the future as a result of climate change and cropping system intensification [4]. The usage of nematicides is being curtailed due to growing concerns about human health and the environment, which has resulted in their prohibition. Scientists are looking for a different alternative that is eco-friendly and cost-effective for farmers. Some of the proposed eco-friendly approaches for managing plant-parasitic nematodes include the use of biological control agents (BCAs) such as *Trichoderma*, mycorrhizal, and endophytic fungi.

Endoparasitic fungi, nematode-trapping or predacious fungi, parasites of nematode eggs, parasites of nematode cysts, and fungi that produce nematode toxic chemicals are all examples of nematode antagonists. Intriguingly, fungi from a wide range of orders and families can be found in each of these groups. While fungal infections harm plant physiology, mutualistic fungi help hosts defend themselves against pathogens and herbivores. Bioactive substances such as short peptide effectors, enzymes, and secondary metabolites are secreted by fungal partners, which promote colonization and contribute to both symbiotic and harmful relationships [5]. Most important beneficial fungi with biocontrol capacity, such as arbuscular mycorrhizas, ectomycorrhizas, endophytes, *Trichoderma* spp., and yeasts, are among those, which can also be achieved through induced systemic resistance (ISR) induction [6]. In this chapter, we will concentrate on research of *Trichoderma*, endophytic fungi, and mycorrhizal fungi as biocontrol agents for nematode management.

Nematophagous fungi can capture, kill, and digest nematodes. Numerous fungal strains have been examined for antinematode activity, and it has been determined that fungi possess several distinct properties that make them the most effective nematode biocontrol agents. Collagenase, chitinases, and serine proteases are only a few examples of enzymes that can rupture the cuticle of adult nematodes or eggshells, causing significant mortality early on [7]. Proteins, keratin, collagen, and fibres make up the majority of the adult nematode cuticle, while chitin makes up the majority of the nematode egg. As a result, fungal collagenase-mediated collagen degradation is thought to be critical for nematode population control [7]. The fungus uses a mix of mechanical activity and hydrolytic enzymes to breach the worm’s cuticle. The enzymes tear down the cuticle and basic components (protein and chitin) of the eggshells of nematodes.

Arbuscular mycorrhizal fungi (AMF) are a common group of soil microorganisms connected with the phytobiome, belonging to the phylum Glomeromycota. AMF established a complicated relationship with higher plants 400 million years ago in the Ordovician period [8]. Except for most Brassicaceae and Chenopodiaceae species, these obligate biotrophs have beneficial mutualistic relationships with the roots of an estimated 80% of terrestrial plants, including important agricultural crop species [9]. Because of their role as natural biofertilizers, AMF have been pushed as a natural method to preserve and encourage sustainable agriculture. They increase the levels of nitrogen (N), phosphorus (P), and zinc (Zn) in the crop [10, 11]. They serve as bioprotectants against fungal, bacterial, and nematode diseases [12, 13]. They also improve drought tolerance [14].

INTERACTION OF PLANT-PARASITIC NEMATODES WITH DIFFERENT FUNGI

Trichoderma Spp.

The *Trichoderma* genus contains a group of filamentous anamorphic fungi whose teleomorphic condition has been seen in a number of species [15]. *Trichoderma* fungi are characterized by rapid growth and the production of large amounts of conidia, which range in colour from dark to light green. The genomes of three *Trichoderma* species, *T. reesei, T. atroviride*, and *T. virens*, were compared and mycoparasitism was determined to be the genus' primordial method of life, while rhizosphere colonization appeared later. The presence of pathogens in the soil and exudates from plant roots favoured a change toward a more generalist approach in this regard [16]. Under greenhouse circumstances, inoculating tomato seeds with *T. harzianum* lowered the degree of infection caused by the nematode *Meloidogyne javanica*, affecting their establishment, reproduction, and nematode development [17]. They also noticed a considerable decrease in egg hatching, indicating that *Trichoderma* species has significant promise as a biocontrol agent against this nematode. Contina and co-workers [18] studied the fungus-nematode interaction using a GFP labelled strain of *T. harzianum* as a biomarker, demonstrating the nematode's reduced infection and reproduction. Nematode development was inhibited by colonization of *T. harzianum* in tomato roots at various stages of the parasitism, including invasion, galling, and reproduction [19]. The potential of *T. atroviride* to induce systemic resistance in tomato plants against the nematode *M. javanica*, as well as its heredity, was just recently discovered, as was the fact that *M. javanica* resistance was inherited in the offspring of plants treated with *Trichoderma* [20]. Furthermore, commercial formulations of several *Trichoderma* spp. have been shown to induce resistance to *M. incognita* in tomatoes in split-root system tests, with an additive impact on the tomato Mi 1.2 resistance gene [21]. It has raised the prospects of adopting BCAs

CHAPTER 16

Role of Endophytes in the Development of Sustainable Agriculture

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Abstract: Agricultural production is affected by both biotic and abiotic stresses. To increase production to meet the demands of the population (agrochemical products), pesticides are heavily used, which are toxic to the environment as well as to humans and animals, and also very cost-effective. For the development of sustainability in agriculture, minimum use of pesticides is recommended. In this context, microorganisms like endophytic fungi and bacteria are used to promote plant growth and productivity. Endophytic organisms live inside plant tissues and can improve plant growth under normal and challenging conditions. They provide benefits to host plants directly or indirectly by improving plant nutrient uptake, production of phytohormones, targeting pests and pathogens with antibiotics, hydrolytic enzyme production, and inducing plant defence mechanisms. This chapter elaborates on the beneficial uses of endophytic organisms in the agriculture system.

Keywords: Agrochemical, Antibiotics, Endophytic, Environment, Nutrient, Phytohormone, Sustainable.

INTRODUCTION

A development that fulfills the basic needs of present inhabitants and preserves resources for future generations to flourish is included in sustainable development [1]. Agricultural development includes three dimensions of sustainability, namely economic, social, and environmental [2]. The 2030 agenda of FAO is to secure zero hunger, and at the heart of this is sustainable agriculture. To fulfill this target, FAO has many indicators called sustainable development goals (SDG), but agenda 2.4.1 is fully dedicated to it. According to Agenda 2030, target 2.4 is "By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production that help maintain the ecosystem, that strengthen capacity for adaptation to climate change, extreme

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weather, drought, flooding, and other disasters, and that progressively improve land and soil quality".

According to a report of Food Security and Nutrition 2020, hunger continues to increase during the COVID-19 pandemic, and it will add nearly 130 billion people worldwide to chronic hunger by the end of 2020 [3]. There is a lot of pressure on the agriculture sector to reduce hunger and provide healthy food as per the requirements of the population.

Agricultural production is affected by both biotic and abiotic stresses. To increase production to meet the demands of the population (agrochemical products), pesticides are heavily used, which are toxic to the environment as well as to humans and animals, and also very cost-effective. For the development of sustainability in agriculture, minimum use of pesticides is recommended. In this context, microorganisms like endophytic fungi and bacteria are used to promote plant growth and productivity. There are several reports on endophytic bacteria that can promote the growth of many crops like wheat, rice, canola, tomato, potato, *etc* [4, 5]. Fixation of nitrogen, mineral solubilization, production of plant hormones, production of phytochemicals, such as antimicrobial and antioxidant compounds against pathogens and stress conditions, *etc.*, are some of the mechanisms used by endophytic organisms to enhance the growth of a plant.

ENDOPHYTES

Endophytes are defined as mutualistic or symbiotic organisms that reside in plant parts and complete their life cycle in them. The word endophyte is derived from two Greek words. These are Endon, which means "within" and Phytes, which means "in plants" [6]. De Bary [7] introduced the term endophytes and defined them as microorganisms that live inside plant tissues. Endophytic microorganisms are mostly fungal and bacterial in nature, and they are classified into two types based on their relationship with plants: facultative and obligate. Endophytic communities are found in all naturally growing plant species and are not found in any exception to that [8 - 10].

Endophytic Fungi

Endophytic fungi live in mutualistic relationships with the tissues of many plants and produce natural secondary metabolites that belong to flavonoids, terpenoids, steroids, phenolic compounds, alkaloids, *etc* [11]. They are the well-known source of many interesting compounds that belong to an unusual class of natural products (Table **1**) [12, 13]. Most of the endophytic fungi belong to the Ascomycota group that reside beneath the epidermal layer of plant tissue without showing any symptoms of infection (quiescent infection) [14].

Table 1. Drugs Isolated From Endophytic Fungi.

Drug name	Endophytic Fungi	Tree/Plant	Reference
Taxol	*Taxomyces andreanae*	*Taxus brevifolia*	[15]
	Pestalotiopsis microspora	*Taxus wallichiana*	[16]
	Annulohypoxylon sp. (strain MUS1)	*Taxus wallichiana*	[17]
Podophyllotoxin	*Phialocephala fortinii*	*Podophyllum peltatum*	[18]
	Fusarium oxysporum	*Juniperus recurva*	[19]
	Trametes hirsute	*Podophyllum hexandrum*	[20]
	Aspergillus fumigatus	*Juniperus communis*	[21]
	Mucor fragilis	*Podophyllum hexandrum*	[22]
	Alternaria tenuissima	*Podophyllum emodi*	[23]
Camptothecin	*Entrophospora infrequens*	*Nothapodytes foetida*	[24]
	Fusarium solani	*Apodytes dimidiate*	[25]
	Alternaria alternate, *Fomitopsis* sp., and *phomopsis* sp.	*Miquelia dentata*	[26]
	Fusarium solani	*Camptotheca acuminata*	[27]
Vincristine	*Fusarium oxysporum*	*Catharanthus roseus*	[28]
Vinblastine	*Alternaria* sp.	*Catharanthus roseus*	[29]
Both	*Fusarium solani*	*Catharanthus roseus*	[30]
Diosgenin	*Fusarium* sp.	*Dioscorea nipponica*	[31]
	Cephalosporium sp.	*Paris polyphylla* var. *yunnanensis*	[32]
	Paecilomyces sp.	*Paris polyphylla* var. *yunnanensis*	[33]

Endophytic Bacteria

Endophytic bacteria are plant-beneficial bacteria that can be isolated from the surface-sterilized tissue of plants [34]. Most endophytic bacteria belong to a subclass of rhizospheric bacteria that are commonly called plant growth-promoting rhizobacteria (PGPR) [35]. They are found in both above-ground and underground plant parts, but underground parts have the greatest number of these microorganisms [36 - 38]. They help to promote the growth of plants not only by increasing the uptake of nutrients from the soil but also by producing compounds against biotic and abiotic stresses [39 - 41]. Thus, they have some essential traits for the survival of the host plant.

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