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Chapter

Ectomycorrhizal Fungi as Biofertilizers in Forestry

Muhammad Hanif, Zubaria Ashraf, Samar Bashir, Fatima Riaz, Rizwan Amanat, Nousheen Yousaf and Samina Sarwar

Abstract

Ectomycorrhiza (ECM) is an association of fungi with the roots of higher plants in which both the species are equally benefited and appears to be important for the survival of both parties, and no doubt this association offered extensive benefits in the restoration of forest and ecosystem soil aggregation and stabilization. The most important and vital role of ECM fungi, which is analyzed globally, is that they are best and environment friendly biofertilizers. ECM fungi considered as a biotechnological tool in forest management because their role in reforestation, bioremediation, control of soil pathogen, and restoration of ecosystem is reviewed extensively. ECM fungi not only increase the biomass of edible fruiting bodies but also improve soil structure, nutrient cycle, and also produce phytohormones, which increase the growth and survival of seedlings and enhance the photosynthetic rate of plants and also maintain their tolerance level against environmental stresses in forest nursery. Ectomycorrhizas also reduce fertilization expenses in an environment friendly manner. The importance of ECM fungi and mycorrhizal helper bacteria for the growth enhancement of the economically important trees and significantly important role in restoration of sites degraded by forestry operation.

Keywords: biofertilizer, phytopathogens, agriculture, biome, mycorrhization

1. Introduction

Fungi are the most valued and understudied group of microorganisms and are widely distributed in different ecological habitats that inhabit and the consequential requirement to contend against an assorted group of other microorganisms, that is, bacteria, fungi, and viruses also have a strong defense mechanism for survival as many scientists have studied their role industrially, ecologically, and biotechnologically; now, fungal association with trees, which termed as mycorrhiza, is becoming the center of focus for its role in forestry and ecosystem as a biofertilizer. Fungi as biofertilizers are considered the basic core of the ecosystem, and their impact will be felt more in the future; hence, the importance of fungi as biofertilizers cannot be denied. The term mycorrhiza originated from two Greek words “mykes and rhiza,” which mean fungus and roots, respectively, was first coined in 1885 by Albert Bernhard

Frank who was a forest pathologist [1]. Frank also explained that mycorrhiza is an association of ectomycorrhiza (ECM) and roots of higher plant, and this relationship is symbiotic in nature; he further subdivided it into two subcategories, that is, ectomycorrhizal fungi (do not penetrate cortical cells) and endomycorrhizal fungi (penetrate cortical cells). A lot of research has been conducted on symbiotic relationships of fungi and plants by many scientists who showed that approximately 86% of territorial plants get their nutrients through mycorrhizal roots [2]. The number of fungal species that take part in ectomycorrhiza formation is about more than 7000, and, predominantly, species from basidiomycetes class contribute toward ectomycorrhiza formation. In ectomycorrhiza formation, a special network named as Hartig's is a bridge for the metabolic alteration of fungi and the roots through which it plays its role in the mobilization, translocation, and transportation of soil nutrients to the roots of plants through mycorrhizal mantle connection extended into the soil [3].

Many pieces of research also supported an assumption that ectomycorrhiza fungi developed polyphyletic assistance from multiple saprophytic species, as their diversity was initially based on the studies of the reproductive part of fungi, and now they are categorized based on their morph anatomical characteristics. With many significant and viable trees, such as poplar, pine, birch, and oak, ectomycorrhizal associations are being observed [4]. Mycorrhizal fungi (MF) can be helpful for the identification of the structure of the plant community. So, the determination of fungal companion, symbiotic relationship and to understand its structure, function and fundamental importance in ecology terms [5].

2. Role of microbes as biofertilizers

In the recent era, the agricultural sector has mostly relied on synthetic and chemical fertilizers for their betterment, but the excessive use of chemicals has caused various environmental issues, such as increase in temperature, destruction of habitat, unavailability of nutrients due to change in soil structure or profile, and environmental pollution causing health hazards. Hence, researchers are working on different agricultural tools that are considered to be effective or environment friendly with less consumption of energy. Thus, natural ecofriendly microbes (algae, fungi, and bacteria) have been recommended as practicable solutions for extensive agricultural applications economically and also support soil structure as well as various forms of agricultural land, support plant growth by enhancing its nutrient absorbing capacity, and reduce chances of soil-borne diseases [6]. Bacteria as biofertilizer ensure the fixation of atmospheric nitrogen and its availability to plants by synthesizing plant-growth-promoting substances and increasing the solubility of phosphorous. Ectomycorrhizal fungi are an important tool for the absorption of different mineral nutrients such as phosphorus. Therefore, all microbes play an important role in the agricultural sector by providing different services, such as disease resistance, drought tolerance, and increasing and maintaining the nutrient quality of soil [7].

The forest biomes offer vital ecosystem amenities, which include providing habitats for organisms and acting as a sink for different nutrients such as carbon, sodium, and potassium, controlling different harsh factors, such as erosion of soil, extenuating climate change, and manufacturing vital assets, for example, wood timber, fuel, and bioproducts. Owing to different human actions, the efficiency of forests has declined significantly over the passage of time. To overcome the shortage of nutrients and phytopathogens, different chemically synthesized products

are being used in forest development or for diversity enhancement in different sectors. But, this frequently leads to nutrient losses through various factors such as leaching, gaseous losses, and many other harmful factors. The exploitation of biofertilizers instead of relying on different chemicals may increase the growth and development of plant species that enhance the productivity in a more maintainable way. Biofertilizers are mostly used in horticulture and agriculture sectors with less emphasis on forestry. It is mandatory to explore and exploit numerous mechanisms of action, viz., enabling nutrient uptake, phytoprotection, and modulation of phytohormone of biofertilizers that would have supportive role in promoting the ecosystem services of forest biomes. All these factors (i.e., the mechanisms of action of effective microorganisms in biofertilizers, applications of biofertilizers in the forestry sector, and factors influencing the effectiveness of biofertilizer application) will be considered in the given review.

S. No	Groups	Examples	
List of nitrogen-fixing biofertilizers			
1	Free-living	<i>Azotobacter</i> , <i>Beijerinckia</i> , <i>Clostridium</i> , <i>Klebsiella</i> , <i>Anabaena</i> , and <i>Nostoc</i>	
2	Symbiotic	<i>Frankia</i> , <i>Rhizobium</i> , <i>Anabaena</i> , and <i>azollae</i>	
3	Associative symbiotic	<i>Azospirillum</i>	
List of phosphorus-soluble biofertilizers			
1	Bacteria	Some phosphorous solubilizing bacteria are <i>Bacillus subtilis</i> , <i>Bacillus megaterium</i> , <i>Pseudomonas striata</i> and <i>Bacillus circulans</i>	
2	Fungi	<i>Penicillium</i> sp. and <i>Aspergillus awamori</i>	
List of phosphorus-mobilized biofertilizers			
1	Arbuscular mycorrhiza	<i>Glomus</i> sp., <i>Gigaspora</i> sp., <i>Acaulospora</i> sp., <i>Scutellospora</i> sp. and <i>Sclerocystis</i> sp.	
2	Ectomycorrhiza	<i>Laccaria</i> sp., <i>Amanita</i> , <i>Pisolithus</i> sp. and <i>Boletus</i> sp.	
3	Ericoid mycorrhiza	<i>Pezizella ericae</i>	
4	Orchid mycorrhiza	<i>Rhizocotina solani</i>	
S. No	Name	Crop suited	Benefits
List of commonly produced biofertilizers			
1	Rhizobium strains	Legumes (i.e., pulses, groundnut, soybean)	It can cause 10–35% increase in yield 50–200 kg N ha ⁻¹
2	<i>Azotobacter</i>	Soil treatment for non-legume crops (including dry land crops)	Results in 10–15% increase in yield with addition of 20–25 kg N. It is also helpful in control of certain diseases.
3	<i>Azospirillum</i>	Nonlegumes (like maize, barley, oats, sorghum, millet, Sugarcane, rice, etc.)	It causes about 10–20% increase in yield. It can be applied to legumes as a co-inoculant.
4	Phosphate solubilizes two bacterial and two fungal species in this group	Soil application for all crops	5–30% increase in yield
5	Blue-green algae, Azolla	Rice/wetland	20–30 kg N ha ⁻¹ , Azolla can give biomass up to 41–52 tones and fix 32–100 kg N.
6	Mycorrhizas	Trees, crops, and few ornamental plants	It causes 30–50% increase in yield and promotes the availability of Zn, P, S, and water.

3. Fungal biofertilizer and its role in agriculture sustainability

Internationally, agricultural production and food manufacturing has to increase twofold in 2050 or the coming years so as to nourish the global growing population while reducing dependence on conventional chemical fertilizers and pesticides. In the past few decades, the increase in extreme chemical fertilizer and pesticide application for crop production has produced a difference in the soil ecosystem. In the present situation of global warming and a disturbed ecosystem, soil microflora regulates how healthy a generation of plants, animals, and humans would be. In light of this, the usage of fungal biofertilizers as a maintainable solution has gained importance over the years [8].

Pesticides, on the other hand, are a double-edged sword. Though targeted on exact targets, they constrain nontarget organisms including the soil mycoflora, which eases the growth of plants. Research shows how agricultural sustainability and economic stability depend on fungal biofertilizers [9].

4. Mycorrhizal fungi and other soil organisms

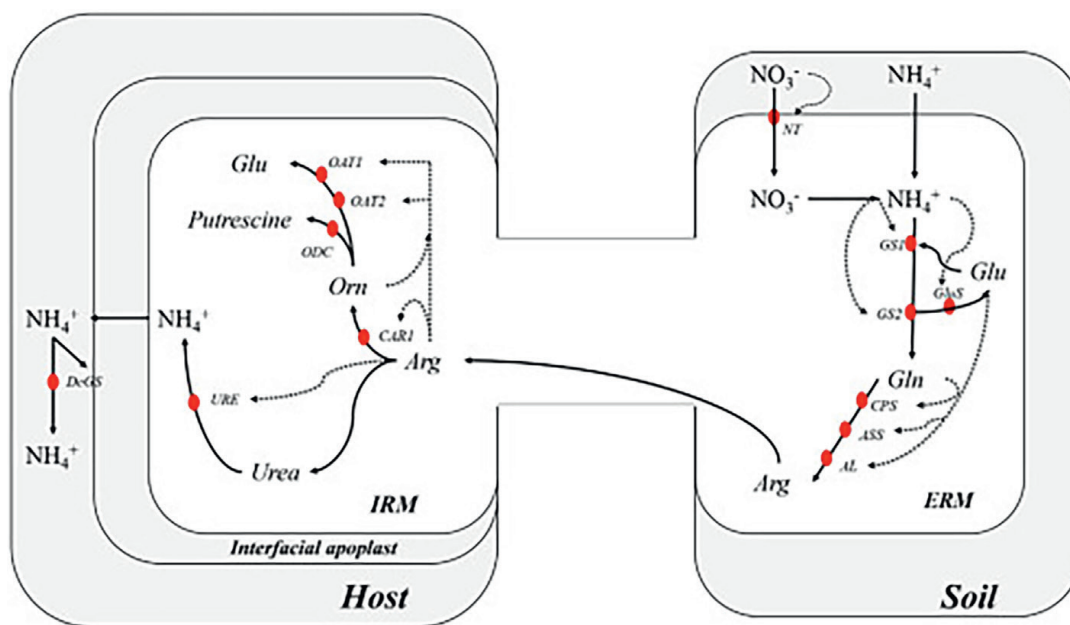
Mycorrhizal fungi interrelate with a vast variety of other soil microorganisms. This interaction may occur in the rhizosphere or in the loose soil. All these interactions may act as an inhibitor or may play a role as a stimulator; most of the time, they play a role of a symbiotic or a competitor. Effects can be observed evidently at all the phases of the life cycle of MF from the spore population dynamic role of mycorrhizal helper bacteria (MHB) in symbiosis interaction; it is very important for bacteria and grazing animals to maintain and sustain this interaction or symbiotic relationship. Mycorrhizal fungi are also capable to amend the associations of plants with other soil organisms—both pathogens, for example, root-inhabiting nematode, fungi, and particularly nitrogen-fixing bacteria. These connections may be significant in natural environment where microbes play regulatory roles, and in agricultural systems, mycorrhizas may be important in the formation of integrated systems of pest control and growth stimulation [10].

5. Mycorrhizas as nutritional mutualisms

Mycorrhiza is a symbiotic relationship between plants and fungi in which both get to benefit from each other; fungi provide all nutrient availability to plants and in return get food or photosynthetic material. The union of many dissimilar systems of mycorrhizas is a testimony to the shared benefits of these enterprises. Resource exchange in mycorrhizas can be observed, and for the inspection of exchanged resources among both partners, different tools are being used. MF is considered more efficient in exploring soil and uptake of nutrients for plants as compared to plant roots. The large size of soil is inhibited by MF that spreads far away from the nutrient reduction zone that develops around roots [11]. About external hyphae of ECM, it is assessed that it may increase the surface area up to 60-fold on average. Nutrients from soil pore spaces are extracted by the small diameter of fungal hyphae that is too small for plant roots to perform its activity [12].

6. Nitrogen fluxes in the plant and fungus symbiosis

Mycorrhizal fungi can use nitrogen in two forms, that is, organic or inorganic, from soil matrix and make their availability to plants. Different research studies carried out by researchers show that ammonium, nitrates, and urea marked with N^{15} . This form of nitrogen is translocated in the hyphae in the form of arginine, though it is quickly converted and then transformed into ammonium ion immediately, while the carbon skeletons created throughout the excruciating of the arginine are reincorporated again into the fungal groups. Now, it is easy to understand how the external hyphae of the ECM incarcerate inorganic nitrogen as nitrate ion, NH_4^+ , and organic nitrogen as amino acids and distribute occasionally a huge fraction of them to the plants.



7. Forest biome and associated microorganisms

Microorganisms are almost ubiquitous in both the terrestrial and aquatic habitats, and regardless of their microscopic proportions, they play an important part in global nutrient cycling. All the multicellular organisms have a close relationship with different microbes, and those organisms that are living in natural environment cannot be considered free from germs. Mutually associated microorganisms use an extensive variety of effects on their hosts from helpful to harmful. Forest biomes are among the species-rich terrestrial systems, and this equally applies to microbes living in forests. Forest soil host not only has rich microbe groups, but also animals and plants that provide them a habitat. Recent developments significantly widened our consideration of the taxonomic and functional variety of microbes living in forest biomes, which can collectively be termed “forest micro biota.” Still, many phases of the dynamics of microorganism values of their assemblage and their contacts with hosts remain unclear and require more research [13].

8. ECM fungi as a source of phosphorous mobilization in forestry

About 97% of phosphorous present in agricultural soil is not available to plants due to its organic or inorganic forms, so fungi are a major source of phosphorous; they provide phosphorous by producing a large amount of acid phosphate, alkaline, and other forms of acid. Different groups of fungi perform these functions and enhance the growth of plants such as *Gliocladium*, *Penicillium*, *Trichoderma*, and *Chaetomium*. Using 45–70 kg phosphorous enhances the yield of different crops by 15–30% with fungi as a mobilizer. The small concentration of organic acid mandatory to mix phosphorous ranges from 0.5 to 0.6%. The influence of microbes was greater in the fungal inoculated plants as compared to plant input. Fungal extracellular enzymes were more effective than their intracellular complement [14].

9. Role of ECM fungi in vegetative propagation in forest nurseries

Vegetative propagation is a vital tool for the enhancement of productivity of economically important horticultural and agricultural plants. Microorganism also plays a dynamic role in the horticultural sector apart from phytohormones, that is, bacteria, fungi, and most important arbuscular mycorrhizal (AM) fungi, because the symbiotic relationship between fungi and plant controls many factors, enhances the ability to endure harsh condition or harsh environmental factors, and triggers hormone production and adventitious root formation [15]. The initial inoculation of arbuscular mycorrhizal fungi onto the medium of roots improves the rate of vegetative-propagated plant species growth after establishing a mutual association with the plant. Furthermore, a series of successive signaling measures are known to happen between AM fungi and plant during the growth and development of roots [7].

10. Ectomycorrhizal fungi and forest restoration

Temperate and boreal forests are affected by anthropogenic activities and destruction. Ectomycorrhizal fungi play a significant role in the re-establishment of forest through building a symbiotic relationship with roots of higher plants of temperate and boreal forests, providing nutrients to their hosts and surface area for gaseous exchange to plant. ECM fungi are involved in woody plant existence and development and help them to tolerate severe environmental conditions [16]. Much research has been conducted on the restoration of forest ecosystems by using ECM fungi in the site where heavy metals and soil erosion destroy plants drastically. The result has proven that ECM fungi restore sites occupied by plant species that are not native. Moreover, boundaries, knowledge gaps, and possible unwanted results of the use of ectomycorrhizal fungi (EMF) in forest reestablishment and proposed for the further incorporation of this fungal group into forest management. Ectomycorrhizal fungi (ECMF) host connections could progress the chances of success of future reestablishment programs in different forests [10].

11. Drought tolerance and ECM fungi

ECMF increase the capacity of plants to bear damaging effects triggered by the shortage of water. This symbiotic relationship affects different metabolic mechanisms

of the host plant and improves the gas exchange mechanism in leaves, photosynthetic rate, and direct assimilation of water from the soil, and allocation to the host plant increases the role of enzymes involved in antioxidant defense, uptake of nitrates, increase application of water through enhanced root hydraulic conductivity osmotic modification, and variations in the flexibility of cell wall. ECMF are useless in severe drought situations [17]. Drought is very common and natural disaster affecting both crop development and livelihood in many ways. It has been assessed that drought damages many plants and crops [18]. Since ECMF have the ability to make the plants which may tolerate water shortage and used to lessen such crop losses. Different research studies have shown that adding ECMF can enhance the growth and production of plants under water-stressed environments. Observations showed that the soil moisture content is higher in ECMF-protected treatments, which shows that ECMF increase soil moisture [19].

12. Disease resistance and ECM fungi

Much research has confirmed that ECMF increase disease resistance in plants against soil-borne plant pathogens. But the efficiency of biocontrol achieved by ECMF is reliant on the species complex, substrate, and host plants. Also, the defense given by ECMF is not effective against all the pathogens controlled by soil and other environmental conditions [20]. This symbiotic relationship protects plants from plant pathogens by using different mechanisms such as changing root development and morphological character creating variations in the tissues of host root and also manufacturing biochemical and physiological variations within the host, varying host nutrition, adapting mycorrhizal sphere that affects microbial populations, challenging for colonization spots and foods, and triggering defense mechanisms and parasitism on nematodes [17].

13. Nitrogen-fixating bacteria and ECM fungi

Recently, research has been conducted to assess the outcomes of biofertilizers, that is, the role of bacteria involved in nitrogen fixation and MF collectively on lentils growth under rainfed conditions associated with plants with no application of *Azotobacter* bacteria and mycorrhizal fungi. Results showed highly effective improvement in leaf moisture, increased amount of chlorophyll, reduced protein amount, and increased biomass production and seed production of plants compared with the control plant [18]. The valuable consequence of mycorrhizal fungi and *Azotobacter* applications showed positive results on photosynthesis in which plant growth was enhanced due to better water uptake and nutrient supply mainly under the shortage of water. Results provide an innovative indication about the effects of biological fertilizers on the growth of lentils and biomass production under rainfed environments. The focus should be on increasing the production of lentil in arid and semiarid area [21].

14. The mycorrhiza helper bacteria

Mycorrhiza derived from word “*mycorrhization*” was first given by two scientist Duponnois and Garbaye in 1991. They described that these bacteria are helpful in the

formation of the plant root and fungus symbiotic relationship. MHB helps to increase the efficiency of mycorrhizal fungi, that is, absorption of different nutrients from soil, and to protect plant roots from pathogens attack and enhance the capacity of plants to uptake a growth factor. This would certainly offer a new measurement of the ecology, evolutionary, and physiology biology of mycorrhiza association. MHB may increase the efficacy of fungal inoculum with a less budget because bacteria are easily grown in profitable numbers than most MF. This research shows that more mycorrhiza helper bacteria work should be devoted to mycorrhizal fungi for commercial concerns as well as used as a laboratory models in research. These fungi include *Pisolithus* spp., *Laccaria bicolor*, and *Glomus intraradices* genomes that are being sequenced as arbuscular fungi ectomycorrhizas. In addition, growing concern about the pollution of soil and the resulting tendency toward reducing the input of chemicals in plant production should substitute eco-friendly practices such as controlled microbial bioremediation, for example, by using ECM fungi as carriers of depolluting bacteria [22]. Different investigation and research supported by the genomic development may be a great opportunity to place MHB in the top prior list of future mycorrhiza research and to increase general field of fungal-bacterial interactions in ecosystems.

15. Role of ECM fungi in the agriculture sector

Uses of different chemicals for acute and chronic disease inhibition may cause environmental pollution and major human health problems [23]. For the sustainability of agriculture, different methods are established by fungal species [21]. Members in the genera *Alternaria*, *Aspergillus*, *Chaetomium*, *Fusarium*, *Penicillium*, *Serendipita*, *Phoma*, and *Trichoderma* are commonly identified as plant development stimulating fungi and also play a vital role as biofertilizer [24]. Different research studies have proven that fungi act as opponents and destroy soil-borne plant pathogens and help in the control of plant disease [25]. Entophytic fungi can be efficiently used as plant defenders, growth stimulators, and competitors of microorganisms, which have capacity for consumption in an extensive diversity of medical agricultural and industrial fields. This is mostly due to their abundant dispersion as symbionts with plants. ECMF recover soil structure, and nutrient source defends plants against root pathogens and also enhances plant growth by making different plants hormones, which may increase the growth rate and also enhance the photosynthesis rate of plants [26]. These fungi are important for the growth improvement of commercially important crops including different trees from the genera *Castanopsis*, *Dipterocarpus*, *Eucalyptus*, *Fagus*, *Picea*, *Pinus*, *Quercus*, and *Shorea*. *Scleroderma* and *Thelephora* are famous ectomycorrhiza genera that upsurge the rate of existence and growth of eucalyptus, oak, and pine seedlings in reestablishment and replantation programs [24].

16. Conclusion

The ectomycorrhiza group of fungi mainly belongs to Basidiomycota and Ascomycota members, and higher plants have mutual association. Different nutrients and water are translocated, absorbed, and utilized by roots of plants; ectomycorrhizal fungi are frequently involved in it. Most of the identified species of eatable fungal are saprophytes, and few are ectomycorrhizal fungi, and in the past few years, ectomycorrhizal fungi has been a topic for research because the role of ectomycorrhizal fungi

in the ecosystem is unbeatable; ectomycorrhizal fungi play a vital role in different environments, for example, in terrestrial ecosystem and frequently involved in nutrient cycling, that is, universal carbon and nutrient cycles in the fungus plant interface the role of carbon and nutrient seems significant. Researchers should focus on molecular and functional tools involved in relationship between fungi, plant, and soil. For eras, scientists were considering that ectomycorrhizal fungi are only effective in forest ecosystem, but latest research has shown that ectomycorrhizal fungi play an important role in all the environments, but further investigation is required to specify its role in other environments; some examples justify that ectomycorrhizal fungi should be considered a basic tool in ecosystem restoration as in the nursery the application of ectomycorrhiza is very important, to yield ectomycorrhizal forest seedlings and also has vital impact in forest restoration. Now, scientists should focus on identifying suitable tools for the viable methods of development and increase in mycorrhizal fungi application on large scale under maintained environmental conditions. The part of ECM as biofertilizers in bioremediation and biocontrol in reestablishment, replantation, and environmental renovation has been important so far, and its significance role in the sustainability of the environment can be huge, enhancing the acceptance of plants against living and abiotic stress. The role of ECM fungi in present drawbacks of ecosystem, such as the vulnerable decline in plants such as oaks and pines and the process of plant remediation of polluted soils, seems favorable. Further research is going on to choose a new group mutual association for terrestrial reestablishment and replantation.

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