

## SIGNIFICANCE OF ECTOMYCORRHIZAE IN FOREST ECOSYSTEMS OF INDIA

C. Sandeep<sup>a\*</sup>, V. Mohan<sup>b</sup> and Syam Viswanath<sup>a</sup>

<sup>a</sup>Tree Improvement and Genetics Division, Institute of Wood Science and Technology, Bangalore -560003

<sup>b</sup>Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore-641002

\* Corresponding Author, e-mail: sandeep.c.naidu@gmail.com, Mobile: 9886778079

**ABSTRACT:** The biodiversity of Indian forest ecosystem is under serious threat from the last two decade and the endemic plants of Western Ghats, Eastern Ghats as well as few important keystone species which also form climax species in Himalayan regions are facing elimination due to habitat loss. Causes for rapid depletion of forest ecosystem in India has been due to over exploitation, overgrazing, poor regeneration, widespread logging, and rapid increase in human population, tourism, removal of leaf and wood litter from the forests floor, demand for fuel wood, fodder, timber and diversion of forestlands for agriculture. Intensive or extensive inventorying and monitoring “hot spots” of biodiversity has thus become a difficult task to achieve for Indian foresters and ecologists. Ectomycorrhizal diversity with important tree species of India is still in the exploratory phase. Any amount of ectomycorrhizal association in tree seedlings is hence needed rather than no ectomycorrhizal association and some species of ectomycorrhizal fungi can be more useful to trees, in certain geographical and environmental conditions, than others. Therefore these fungal species should be effectively managed and applied.

**Keywords:** Western Ghats, Ectomycorrhizal fungi, Endemics, Exotics, Keystone and Climax.

### INTRODUCTION

This paper explains ecological benefits of mycorrhiza, its role in eco-restoration of forests, inoculation of mycorrhiza in nursery and application of modern biological tools to study biodiversity of fungal symbiosis in forest trees. Let me begin this review with an old story about a man when returning home, one night found his neighbour curiously searching the ground beneath a street lamp. He asked his neighbour why he was so curiously looking into something. The neighbour replied by saying that he had lost his key. The man then asked his neighbour did he know where exactly he dropped had dropped the key? Yes, replied the neighbour, pointing towards far dark corner of street. If at all you have dropped it somewhere in the corner then why you are looking here, questioned the man. “Because this is the actual place where the light is” answered the neighbour. By this story we can say that the task of the ecologist is not to bring the search to where the light is, but to bring the light to where the search exactly is [1]. India, one among 12 mega diversity countries across the globe consists more than 8% of the world's total biodiversity and its biogeographic ecosystems is classified into ten zones such as Western Ghats, Deccan Peninsula, Gangetic Plain, Desert, Semi-arid, Trans-Himalaya, Himalaya, Coasts, Northeast and Islands [2].

The term ‘mycorrhiza’ (*Gr. Mykes=Fungus or Mushroom; Rhiza=Root*) i.e ‘fungal root’ coined by Frank [3], is a non-pathogenic mutualistic symbiotic association between soil bound fungi with the roots of higher plants. It obtains organic nutrition (carbohydrates, vitamins, amino acids and plant growth substances) from plants and also perfect ecological niche that is necessary for fungal growth and development including the completion of the sexual cycle. Mycorrhizal associations sometimes may influence both biodiversity [4] and biogeochemistry [5].

The benefits to its host plants include faster growth, increased uptake of essential nutrients such as phosphate and inorganic nitrogen, improved tolerance to biotic and abiotic stress, survive transplantation shock along with resistance to plant pathogens and invasion by weeds, synergistic interaction with other beneficial soil microbes and many number of genes required for symbiosis beneficial alterations of plant growth regulators and improved soil structure for better aeration and water percolation [6]. These symbiotic fungi are also economically important as edible mushrooms and as mutualists of trees planted for forestry [7]. Due to their wide beneficial properties, the mycorrhizal fungi should be considered as an integral part of normally functioning root system in symbiosis and function as effective biological linkages.

### Classification of mycorrhizae

Mycorrhizae previously were classified as ectomycorrhizae and endomycorrhizae, mainly upon the structure of fungus root association and later renamed as ectotrophic for ectomycorrhizae and endotrophic for endomycorrhizae. Mycorrhizae are now classified into seven types, (Figure 1) (Vesicular Arbuscular, Arbutoid, Ectendo, Ecto, Ericoid, Monotropoid, and Orchidaceous mycorrhiza). Among them, the widely described are arbuscular mycorrhizal (AM) fungi (Glomeromycota) and ectomycorrhizal (ECM) fungi (Ascomycota and Basidiomycota) which are the most abundant and widespread in forest communities [8].

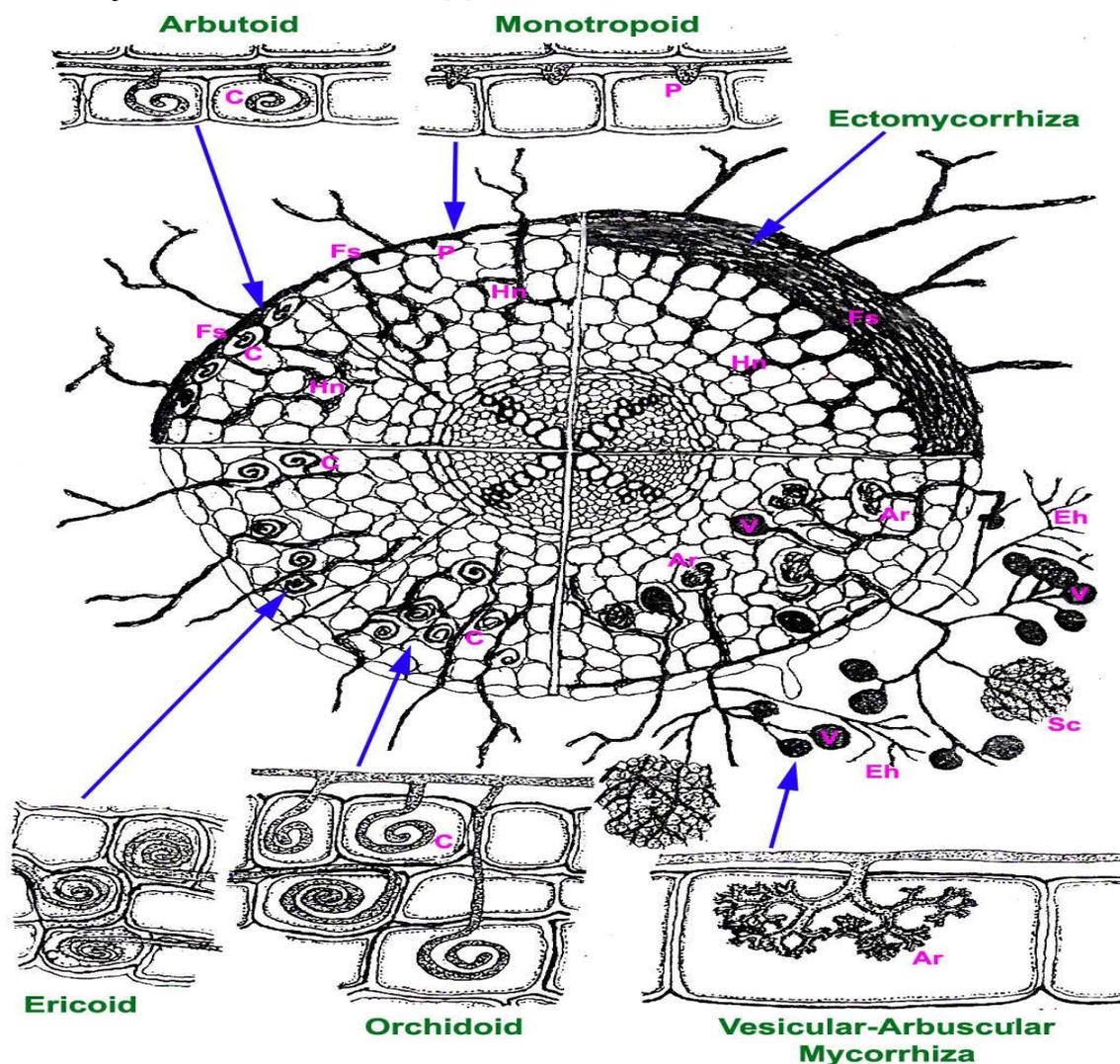


Figure 1: Different types of Mycorrhiza (Adapted from Mycorrhizal Biology by K.G. Mukherji, B.P. Chamola and Jagjit Singh). Ar = Arbuscules, C = Coiled hyphae, Eh = Extrametrical hyphae, Hn = Hartig net, Fs = Fungal sheath, P = Protrusion, Sc = Sclerotia, V = Vesicles).

**Ectomycorrhizae (ECM):** It is an association of fungus and feeder roots (root hairs) in which the fungus grows predominantly intercellularly in cortical region penetrating the epidermis by secreting proteolytic enzymes and develops extensively outside the root forming a network of hyphae called as 'Fungal sheath' or 'Fungus Mantle' which is of variable thickness and color. There is an intercellular infection forming a network of fungal mycelium around the cortical cells called 'Hartig-net'. The 'Hartig-net' structures serve as the centers for nutrient exchange between the host plants and the fungus. Nutrients and water have to pass the fungal mantle. In general, the extensive mycelium facilitates more efficient growth for the phycobiont due to improved nutrient and water uptake. Root hair morphology and color differ in different environments and ECM colonization does not spread beyond the endodermis or into meristem tissues and this type of association is common in temperate region, tropical region to boreal forest trees. In India, the number of host species increases with altitudes and higher latitudes. Over 5000 fungi belonging to Basidiomycetes, and Ascomycetes involved in forming ECM on about 2000 plants have been identified worldwide. Plants belonging to families, *Dipterocarpaceae*, *Mimosaceae*, *Cistaceae*, *Pinaceae*, *Fagaceae*, *Betulaceae*, *Salicaceae*, *Juglandaceae*, *Myrtaceae*, *Ericaceae*, *Ulmaceae* and a few others commonly form ECM associations.

#### **Characterization and identification of ectomycorrhizae**

The data on ECM diversity in many parts of India is still not sufficiently documented. The conserved characteristics of its structure such as its tissues, the arrangement and organization can be used to describe ectomycorrhizal fungi in the same way as any other taxonomically suitable feature. The goal of elucidating the structure and development of mycorrhizal communities remains still unexplored in India. The ECM can also grow their hyphae within the rhizomorphs and ECM of other fungi, suggesting that these fungi can influence each other with respect to plant nutrition and with respect to their fruit body formation. Community structure characterization can be done by amplifying DNA using fungus specific primers followed by digestion with restriction endonucleases to get restriction fragments patterns of a taxon. Benefits of molecular tools include development of better and innovative sampling strategies, observation of host specificity in field, exploration of dynamics in replacement processes, and mainly important is the determination of the dispersal process involved in community development. With the introduction of new additional techniques; population analysis, questions related to the resolution of genetic structure and variation, gene flow in important ECM communities in India have to be studied indepth. Riviere, Diedhiou, Diabate, *et al.* [9] studied genetic diversity of ECM basidiomycetes from African and Indian tropical rain forests and concluded both types were genetically diverse.

#### **Cultural characteristics**

Macro and microscopic features of ECM fungi, with regard to their particular importance as possible taxonomic criteria should be used for identifying diverse range of ECM communities in India. Lakhanpal [10] has recorded 72 species of fungi belonging to families *Amanitaceae*, *Agaricaceae*, *Hygrophoraceae*, *Tricholomataceae*, *Russulaceae*, *Strophariaceae*, and *Paxillaceae* to be mycorrhizal with different trees in north-western Himalayas. Sharma and Mishra [11] recorded maximum colony growth in *Laccaria laccata* at pH 5 on MNM (Modified Melin Norkran's) culture medium. *Collybia radiata* grew at pH 6 and *Pisolithus tinctorius* at pH 7. Different culture media such as Modified MN medium, NM (Norkran's medium) and HM (Hagem's medium) were tested to find a suitable medium for mass multiplication of *P. tinctorius*.

#### **Use of modern techniques for recording ectomycorrhizal fungi in forests**

Studies of ECM in forests recorded by sporocarp studies or by morphological identification of ECM root tips are based on descriptions of ECM morphotypes by Agerer [12], Ingleby *et al.* [13], Goodman *et al.* [14] Mohan *et al.* [15, 16 and 17], is traditional and can be difficult to distinguish ECM using these methods, because many described morphotypes have not been identified to species level, and because different species may have similar morphology, such as ECM mantle structure which can lead to misidentification of some morphotypes [18]. Assessments of ECM diversity based on sporocarps alone usually cannot adequately describe ECM communities, because many species may sporulate irregularly or not at all. There may in fact, be little correlation between above- and below-ground ECM communities; species that are poorly-represented underground on roots may sporulate prolifically and may be over-represented above-ground as sporocarps, and vice versa [19]. Morphological characters of ECM are usually not sufficient for species recognition, especially in tropical habitats where many species coexist but remain poorly known, though not totally unknown. In such a context, molecular tools can prove to be of great help for ecology, systematic and ECM fungal diversity assessment. PCR-based methods help to overcome many of the recent problems, and has allowed researchers to study the detailed diversity, distribution and ecology of ECM communities. Ray and Adholeya [20] developed molecular markers for four heavy-metal tolerant isolates of *Laccaria fraterna* and *P. tinctorius*. Combining molecular methods with traditional morphotyping may be the most time and cost effective method of garnering information about the below-ground communities in forest ecosystems.

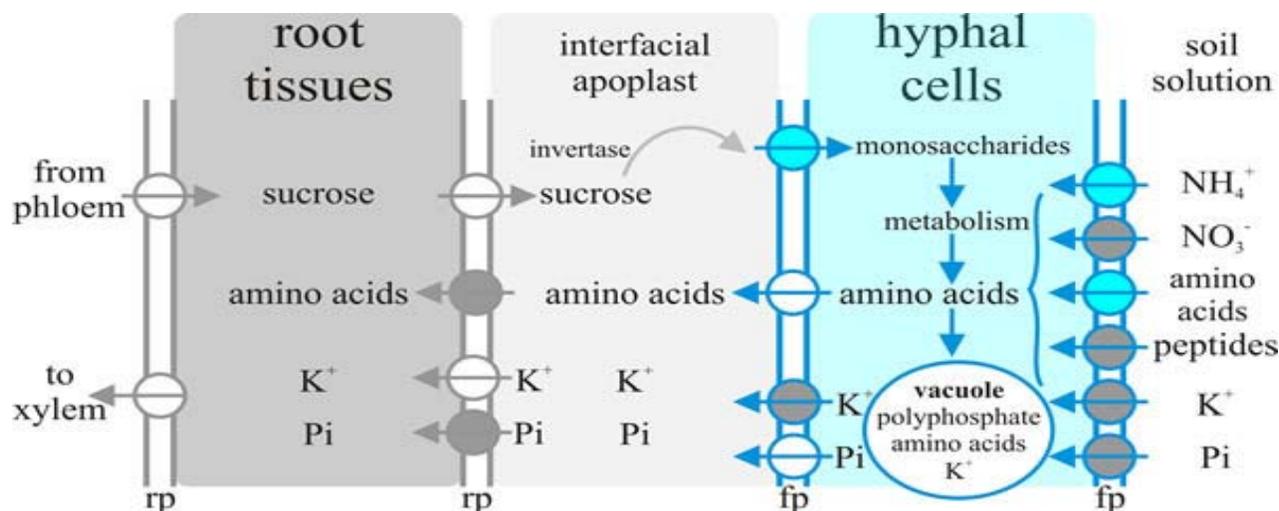
Recent advances in ECM identification in small environmental samples of tree roots by attenuated total reflection (ATR) fourier transform infrared (FTIR) spectroscopy was done by Pena *et al.* [21]. Therefore, combinations of morphological and molecular methods can be used to reveal extremely high ECM fungal species richness.

### Ecological benefits of Ectomycorrhizae

ECM differs in their ability to associate with different host species. Some fungus species is restricted to specific genera. Some fungi may be able to form ectomycorrhizae with wide range of host species which may be limited in distribution by habitat requirements. Hence their interactions with host and habit requirements are not well understood, especially in Indian forest ecosystem. In India, the ECM associations are commonly found in Himalayan region, Western and Eastern Ghats and also in the states of Uttar Pradesh and Madhya Pradesh. The Himalayas is divided into three zones based on altitudinal variation, *viz.* Sub-Tropical, Temperate and Alpine [22] from dry tropical deciduous in the foothills to the alpine scrub forest at timberline. The keystone species such as oak (*Quercus* spp.) which forms climax species in Himalayan regions are facing elimination due to habitat loss. The Eastern Ghats are located between 11° 31' and 22° N latitude and 76° 50' and 86° 30' E longitudes in a North-East to South-West strike and covers an area of 75,000 Sq. km with width of about 200 km in the North and 100 km in the South. It is a rich source of biological species, geological formations and ethnic groups. Over 2,600 plant species of pteridophytes, gymnosperms and angiosperms including 160 species of cultivated plants have been documented in this region. Eastern Ghats of India are spread over three states, namely Orissa, Andhra Pradesh and Tamil Nadu. The depletion of forest cover in this region is due to population explosion and mining related activities. The *Acacia-capparis* belonging to Deccan plateau dry regions of Telangana, Karnataka, and the plains west of the Aravalli hills in Rajasthan, has been wiped out except for small Maharashtra, Saurashtra in Gujarat, patches like the Velavadar National Park in Gujarat. The thorn forest of the semi-arid zones of the Deccan Plateau used to be covered by *Acacia nilotica* and *Anogeissus latifolia*, but today it is eliminated and the forests of northern Uttar Pradesh and many parts of the Himalayas is severely threatened. The Western Ghats is 1600 km long mountain range, with an elevation rising up to 2800 m, variable breadth of 5-25 km, lying parallel to west coast of India which has variety of forest types ranging from evergreen, semi evergreen, deciduous forests, *Acacia* forests to shola and grasslands. The common ECM fungi *viz.*, *Cantharellus*, *Laccaria*, *Russula* and *Amanita* which are associated with endangered endemic local tree species of Western Ghats such as *Acacia* spp., *Myristica malabarica*, *Hopea parviflora*, *Vateria indica*, *Dipterocarpus indicus* and *Terminalia paniculata*. The ECM fungal diversity in local regions of Eastern and Western Ghats as well as in Himalayan regions is poorly understood. The function of ECM in the forest ecosystems has to be concentrated rather than studies related to its symbiosis. Significance of ECM in Dipterocarps forest ecosystem is enormous and its management is very important. Dipterocarps are tropical forests species whose seedlings can survive and grow under very low light intensities, and require ECM associations. Plants have been grouped into three categories based on their degree of association with mycorrhizal fungi such as Non-Mycorrhizal, Facultative and Obligatory [23]. The ECM associations with roots are affected by its abiotic and biotic factors, as well as anthropogenic perturbations. Low phosphorous and lack of season in a tropical soil may be the reason for tropical species to depend on mycorrhiza for survival and growth. Exotics have been introduced on a large scale, and the demand for Casuarinas, Pines, Eucalypts and Bamboo is large because they are fast-growing and profitable for farmers but these exotics at present is plagued by several diseases and loss of good quality timber yield. Hormone relationships, induced by fungal symbionts make ECM roots have greater longevity (length of physiological activity) compared to non-mycorrhizal roots. Other than its role in exotic plantation establishment, symbiotic fungal association can be more useful in eco-restoration of degraded forests, which is now regarded to be one of the prioritized research problems.

**1. Plant Nutrition:** ECM absorbs and stores plant nutrients like nitrogen, phosphorus, potassium and calcium etc in their mantle thereby help in better crop stand, establishment of high yielding forests, land reclamation and establishment of exotic plant species. Influence of ECM on root exudates is important for the nutrient exchange and mineralization process in the soil [24].

The nutrient sorption by the plants is by release of exudates in the form of low molecular weight carboxylates, which in turn mobilize the required elements and form organ metallic complexes that are in a form suitable to be taken up by plants for metabolic purposes [24].



**Figure 2: Nutrient exchange between fungus and host in the ectomycorrhiza depends on one partner releasing nutrient into the apoplastic interface and the uptake of that nutrient from the interfacial apoplast by the other partner and transporters acting in ectomycorrhizal tissues that achieve this nutrient exchange (adapted from Moore *et al.*, 2011). Blue circles represent transporters where at least one member of the transporter family has been characterized by functional complementation of a yeast deficient strain; grey circles are putative transporters for which candidate genes exist in the genome; white circles represent hypothetical transporters.**

Studies conducted so far says that nitrate ( $\text{NO}_3^-$ ) is more mobile than ammonia which is main source of the nitrogen in agricultural soils and usually plants will naturally take up ammonia over nitrate and it has been shown that many of the ECM fungi may not be able to utilize nitrate which is advantageous to plants even though the ammonia is present in low concentration, the ECM fungi will be able absorb it more rapidly than nitrate. However, an important consideration is nitrate and ammonia salts which are subject to constant leaching by rain water and this effect will keep their concentrations relatively low in the rhizosphere of most natural soils. It is also very important that ECM and ericoid mycorrhizas can utilize organic N sources by producing acid proteinases. This will allow them accession of N sources that are otherwise unavailable to the plant roots. On the contrary arbuscular mycorrhizal (AM) inoculation has little profound effect on N nutrition especially when nitrate is abundant in the soil and AM plants have no advantage over non-AM plants. The above study and thumb rule depicted by Moore *et al.* [24] explains that ECM and ericoid plants usually dominate in N-limited soils, and AM dominate in P-limited soils.

Bi-directional movement of nutrients characterizes the mycorrhizal symbiosis. Carbon flows to the fungus and inorganic nutrients move to the plant which acts as a critical linkage between the plant root and the soil. Nutrients taken up by the mycorrhizal fungi in depleted soils can lead to improved plant growth. Thus it can be said that mycorrhizal plants are more competitive and better adapted to tolerate environmental stresses than non-mycorrhizal plants. The uptake and exchanges of nutrient and metabolites at biotrophic interfaces are controlled by the activity of transporters located in the fungal or the plant membrane. Therefore, their patterns of regulation are essential in determining the outcome of plant fungal interactions and in adapting to changes in soil nutrient quantity and quality [25-26].

**2. Stress Tolerance:** ECM association association's helps plants to overcome different kinds of stress such as soil salinity, alkalinity and acidity, drought conditions which may be due to exploitation of larger soil volume, extended root growth and increased absorptive area. The ECM plants exhibit better growth than the non mycorrhizal ones especially in the arid and semi arid regions where low moisture and high temperature are very critical for survival and growth of the plants.

**3. Heavy Metal Tolerance:** ECM plants are also more tolerant to toxic heavy metals than the non- mycorrhizal plants. Many human activities, such as ore mining and smelting, sewage sludge treatment and fossil fuel consumption, result in toxic soil concentration of heavy metals (Aluminum, cadmium, chromium, cobalt, copper, mercury, manganese, nickel, lead, tin, zinc and others). There are also natural soils with levels of heavy metals that inhibit or preclude the growth of many plants and soil microorganisms. However, certain plants and microorganisms do grow in metalliferous sites by the process known as amelioration of toxicity. Various fungi are known to interact with heavy metals differentially according to their binding ability and their tolerance.

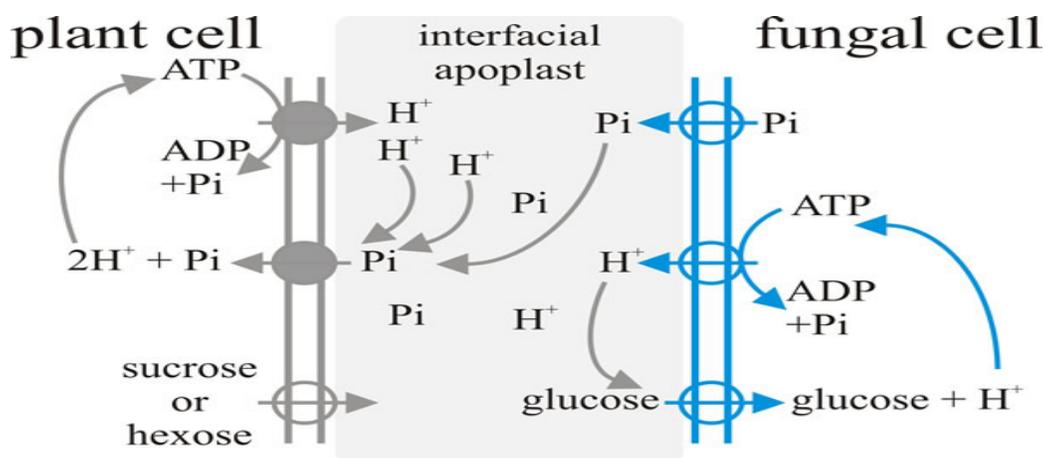
ECM fungi act as a phytoextractor and efficiently transfer nutrients to the host plant, while simultaneously blocking the transfer of the heavy metals. In this way, these ECM fungal species are capable of protecting the host from the toxic metals. ECM fungi growing in polluted soils often exhibit heavy metals in their sporocarps in addition to other mycelial tissue. The detoxification in ECM fungi usually takes place by binding of heavy metals onto the cell walls and accumulation in their vacuoles. Heavy metal binding to the cell wall by using components such as melanin and chitin may also play crucial role in the mechanism of heavy metal tolerance by ECM fungi. It is presumed that heavy metal binding into the extrametrical mycelium is crucial for the ameliorating effects of the mycorrhizas. There are numerous reports of the isolation of metal tolerant strains of ECM fungi from polluted sites. The effect of heavy metals such as chromium and nickel growth and formation of ECM was studied by Aggangan [27] and he reported that the isolate from heavy metal contaminant site, not only colonized greater percentage of root tips, but it was more effective in promoting seedling total biomass in chromium amended soil than other isolates. Therefore more in-depth studies are needed to utilize heavy metal tolerant ECM fungi for phytoremediation in different heavy metal contaminated soils.

**4. Carbon Transfer:** ECM fungi behave as a sink of photosynthetic products, produce hormones that influences the translocation of carbon compounds and convert the host sugars into storage sugars. The excess carbon accumulates in patches and towards the edge of hyphal mats, and supplies energy to detrital food web thus benefiting saprophytic microbes and other plant growth promoting microorganisms. Hence the role of ECM in carbon allocation of the forest ecosystem is a very important process. ECM link multiple trees of different chronological stages by common mycorrhizal networks [28] (CMN) and these linkages may facilitate carbon transfer between connected trees, allow for the absorption of limiting nutrients and water by connected trees. These two functions of CMN promote structural heterogeneity in forest ecosystems, by allowing young seedlings to survive in the presence of much larger trees. Therefore, ECM association with forest trees improves the absorption of almost all the nutrients required by them for their growth and development.

**5. Growth Hormones:** Plants with mycorrhiza exhibit higher content of growth regulators like cytokinins and auxins as compared to the non-mycorrhizal ones. Growth hormones produced by ECM fungi, it is evident that benefits to the higher plants provided by the fungal symbiosis is not just limited to inorganic and organic nutrients from the soil. The fungal symbiont provides the host plant with growth hormones, including Auxin, Cytokinins, Gibberlins and also growth-regulating B-vitamins. Since these hormones are homologous to those formed endogenously by the host plant, the latter may have excessive amounts of hormonal regulators. The above normal levels of these potent substances influence the growth and development of host plant.

**6. Utilization of fixed phosphates and insoluble phosphates:** Phosphorus (P), predominantly in the form of phosphate ( $\text{PO}_4^{3-}$ ) is a key element to all life forms, building blocks of genes and chromosomes. In addition to providing the phosphate ester backbone of DNA and RNA, P is critical in transmission of chemical energy *via* the ATP molecule and in structural components such as phospholipids in cell membranes (Figure 3). Phosphorus is the second most important element after nitrogen that plant requires and is involved in various processes such as cell division, photosynthesis, biological oxidations, transfer of energy and nutrient uptake by crop plants. Symbiotic mycorrhizal association can lead to more economical use of phosphate fertilizers and better exploitation of cheaper and less soluble rock phosphates. Production of phosphatases by ectomycorrhizal fungi plays very important of solubilization of organic phytates. The ECM fungi have been shown to produce large amounts of calcium oxalate, which may be involved in the chelation of Fe and Al and, thereby release P for plant uptake [29]. Some ECM fungi are specialized in the release of Pi from insoluble mineral P by excretion of low molecular weight organic anions such as oxalate but the relative contribution of insoluble P dissolution *in situ* remains unknown. Also phosphatase released from mycorrhizas is likely to play a significant role in acquisition of Pi from labile organic forms of P (Po). In general, these microorganisms cannot access the major source of soil P directly but first bioconvert them to either soluble ionic phosphate.

**7. Protection of plants from attack of pathogens:** Objective of forestry is developing and protecting forests for their maximum productivity. With increasing demand for wood, fodder, fuel and timber, plantation forestry has gained importance in recent years. Diseases can significantly diminish the growth and yield or reduce the usefulness of a plant or plant product. When plants are severely affected by continuous irritation of disease-causing agents which interferes with their normal development and functioning, they are considered to be diseased. They are grouped based on causal agent involved (deficiency diseases, fungal diseases, bacterial diseases, viral diseases, nematode diseases, etc.), the plant part affected (root, seedling, leaf, stem, flower, fruit, tuber etc.) or the type of symptoms (damping-off, wilts, leaf spots, cankers, blights, galls, root-knots, mosaics, storage rots, etc.).



**Figure 3: Model for the exchange-transfer of phosphate and carbon compounds across the arbuscular mycorrhizal interface. Plasma membrane proton-pump ATPases and secondary transporters that have been experimentally localised in the membranes of the arbuscular interface are indicated by the circles, with the arrows indicating direction of transport (Adapted from Moore *et al.*, 2011).**

Wide range of fungal and bacterial pathogens attack seedlings, saplings and trees of Albizia, Neem, Pongamia, Eucalypts, Casuarinas, Teak and other economically important tree species. Hence, it is essential to identify the various diseases of important tree species in nurseries and plantations and develop suitable management strategies for their better productivity. Mycorrhiza is found to offer adequate protection to the root system from the attack of pathogenic fungi. The ECM fungi such as *Lactarius deliciosus* and *Boletus sp.* antagonize *Rhizoctonia solani*. *Lactarius camphorates*, *Lactarius sp.* and *Cortinarius sp.* have been found to produce antibiotics known as 'chloromycorrhiza' and 'Mycorrhizin A' which are antifungal to the phytopathogens like *Rhizoctonia solani*, *Pythium debarynum* and *Fusarium oxysporum*. Besides the fungal mantle in the mycorrhizal roots also offers physical resistance to various soil borne pathogenic fungi if the mycorrhizal fungus gains entry into root system prior to infection of root by the potential pathogen. In pine seedlings, the fungus mantle has been found to restrict the penetration of *Phytophthora cinnamomi*. Moreover some insoluble polysaccharides are known to accumulate in the cell wall and lignin production is enhanced in the mycorrhizal roots. In such tissues the growth of pathogens like *Fusarium oxysporum* and *Pyrenochaeta terrestres* have been found to be considerably restricted. It has now been well established that inoculation of mycorrhizal fungi particularly the ECM ones can protect the roots from soil borne pathogens and nematodes by forming a sheath around the roots and stimulate the plant growth by reducing the severity of diseases.

**8. Soil Aggregation and soil structure:** It is a complex process, hierarchically structured one, in which numerous organisms and binding agents play a role, as well as abiotic factors (such as wetting- drying and freeze-thaw cycles). ECM fungi can play an important role in aggregate stabilization and studies have to be carried out to understand the process of aggregate stabilization by these symbiotic fungi. ECM fungi has a major impact on soil structure because of the wide spread mycelial mats over large area of soil and yet there is very less literature available on soils of Indian ecosystems.

**9. Edible Mushrooms and animal food:** Relationship between truffle-like fungi, vertebrates such as squirrels and many ground-dwelling marsupials, and the host trees, are well known but role of ECM fungi as food source for invertebrates and role of invertebrates in long distance dispersal of sporocarps have to be studied in Indian forest ecosystems and the process of mammal mycophagy has to be understood. In South East Asia, sporocarps of basidiomycetes are collected for local consumption and trade. The highest diversity of fungi can be recorded in Indian forests because of its diverse climatic range and these fungi have high medicinal value, for their aesthetics and as bio-indicators of environmental quality.

**Methodology for inoculation of Ectomycorrhizae:** It can said that any amount of ECM association in tree seedlings is primarily needed rather than no ECM association and some species of ECM fungi can be more beneficial to trees, under certain environmental conditions, than others. Field performance of tree seedlings is improved by forming ECM on them in nurseries with specific fungi ecologically adapted to the planting site. Now it is well known that trees planted on highly disturbed areas receive high mortality unless given the proper mycorrhizal fungus.

The need of pine and oak seedlings for ECM has also been convincingly demonstrated in the afforestation of former treeless areas such as the grasslands of Russia and Great Plains of the US. The beneficial effects of ECM fungi on seedling growth and development in artificially regenerated forest sites and drastically disturbed land have been studied by Marx *et al.* [30]. The primary purpose for inoculating with these specialized fungi in world forestry is to provide seedlings with adequate ECM to improve their survival and growth after planting to create man-made forests. Such treatment has proven essential in forestation of cutover lands and other treeless areas and the introduction of exotic tree species, where native ECM fungi are deficient or reduced in species diversity.

Most research on inoculation with ECM fungi should be based on two working premises. First, any ECM association in tree seedlings is far better than no such association at all. Secondly, some species of ECM fungi have proven to be more beneficial to trees under certain environmental conditions than others. Several types of natural and laboratory-produced inoculants and several methods of application have been used over the years. Many of the techniques have proven successful while others have not. The use of forest soil inoculum has major disadvantages. Species composition of ECM fungi in the soil inoculum is usually not known, and the inoculum may also contain harmful microorganisms and noxious weeds. The use of soil inoculum, however, is consistent with the premise that any ECM are better than none. Pure mycelial or vegetative inoculum of ECM fungi is recommended as the most biologically sound material for inoculation. Several researchers in various parts of the world have developed procedures for culturing vegetative inocula of a variety of ECM fungi for research purposes. Large scale nursery applications of pure mycelial cultures have been severely hampered by the inability to produce large quantities of viable and economical ECM inoculum. It is obvious that in the past many methods were developed to ensure the formation of ECM on tree seedlings used to establish man made forests. Certain methods have advantages over others. Pure vegetative and basidiospore inocula have the greatest biological advantages. Specific ECM fungi have to be actively used in practical reforestation and reclamation programs. Inoculation of containerized seedlings with pure cultures of potential ECM fungi has to be practiced.

## CONCLUSION

Every living organism in an ecosystem is limited to its tasks and therefore an ecological “division of labour” occurs among the organisms in the ecosystem. If one organism disappears then there is a chance that another organism can fill its functional role, but some organisms are key participants to the ecosystem and once lost, their function will be lost as well. This would lead to changes in the ecosystem structure, chemistry and biological characteristics. Hence, ECM fungal association can be regarded as important criteria and phenomenon for conservation and establishment of forest tree species. Research on development of more potential ECM fungal strains capable of ECM association is needed and protocol for their utilization with forest trees have to be standardized. The ECM fungi play an important role through weathering, N-mineralization, biotic and abiotic tolerance and resistance, plant nutrition, also likely in podzolisation and make its host tolerant to various heavy metals through specialized mechanisms. Since, India having wide climatic range, diverse group of ECM fungal species and their distribution over different soil horizons needs to be studied. Assessment and understanding of ECM diversity and its ecology especially in Indian forest ecosystems is highly important as the green cover over the last few decades is overexploited.

## REFERENCES

- [1] Perry D A, Oren R and Hart S. 2008. *Forest ecosystems*. Baltimore, MD: John Hopkins University Press.
- [2] Rodgers W A, Panwar H S and Mathur V B. 2002. *Wildlife Protected Area Network in India: A Review* (Executive Summary). Wildlife Institute of India, Dehardun.
- [3] Frank A B, Uber die and Wurzed symbiose. 1885. Beruhende ernanrung gewisser baume durch unterirdische pilze. *Ber. Dtsch, Bot. Ges.* 3: 128-145.
- [4] Bever J D. 2002. Host-specificity of AM fungal population growth rates can generate feedback on plant growth, *Plant and Soil*, 244: 281–290.
- [5] Hoffland E H, Thomas W K, Hakan W, Plassard C, Anna A G, Kurt H, Rosling A and Breemen N V. 2004. The role of fungi in weathering. *Frontiers in Ecology and the Environment* 2:258-264.
- [6] Ferrol N, Barea J M and Azcon-Aguilar C. 2002. Mechanism of nutrient transport across interfaces in arbuscular mycorrhizas. *Plant Soil*, 244: 231-237.
- [7] Yun W and Hall Ian R. 2004. Edible ectomycorrhizal mushrooms: challenges and achievements. *Can. J. Bot.* Vol. 82: 1063-1073.

- [8] Smith S E and Read D J, Mycorrhizal symbiosis. 2008. 3<sup>rd</sup> edn. Academic Press, London.
- [9] Riviere T, Diedhiou A G, Diabate M, Senthilarasu G, Natarajan K, Verbeken A, Buyck B, Dreyfus B, Bena G, Ba A M. 2007. Genetic diversity of Ectomycorrhizal Basidiomycetes from African and Indian tropical rain forests *Mycorrhiza* 17(5): 415–428.
- [10] Lakhanpal T N. 1987. Survey and studies on mushrooms and toadstools of Himachal Pradesh in North-western Himalayas India: Department of Science and Technology (*Final Technical Report*).
- [11] Sharma G D and Mishra R R. 1982. Mycorrhizal association in gymnosperms of Meghalaya *Acta Botanica Indica* 10: 43–49.
- [12] Agerer R. 1987–2002. *Colour Atlas of Ectomycorrhizae*. Einhorn- Verlag, Munich.
- [13] Ingleby K, Mason P A, Last F T and Fleming L V. 1990. Identification of ectomycorrhizas, ITE research publication no. 5. HMSO, London.
- [14] Goodman D M, Durall D M, Trofymow J A and Berch S M. 1996. A manual of concise descriptions of north american ectomycorrhizae: including microscopic and molecular characterization. *Mycologue Publications, and the Canada-BC Forest Resource Development Agreement*, Pacific Forestry Centre, Victoria, B.C.
- [15] Mohan, V., Natarajan, K. and Ingleby, K. 1993a. Anatomical studies on ectomycorrhizas. I. The ectomycorrhizas produced by *Thelephora terrestris* on *Pinus patula*. *Mycorrhiza*. 3: 39-42.
- [16] Mohan, V., Natarajan, K. and Ingleby, K. 1993b. Anatomical studies on ectomycorrhizas. II. The ectomycorrhizas produced by *Amanita muscaria*, *Laccaria laccata* and *Suillus brevipes* on *Pinus patula*. *Mycorrhiza*. 3: 43-49.
- [17] Mohan, V., Natarajan, K. and Ingleby, K. 1993c. Anatomical studies on ectomycorrhizas. III. The ectomycorrhizas produced by *Rhizopogon luteolus* and *Scleroderma citrinum* on *Pinus patula*. *Mycorrhiza*. 3: 51-56.
- [18] Sakakibara S M, Jones M D, Gillespie M, Hagerman S M, Forrest M E, Simard S W and Durall D M. 2002. A comparison of ectomycorrhiza identification based on morph typing and PCR-RFLP analysis. *Mycol. Res.*, 106: 868-878.
- [19] Durall D M, Gamiet S, Simard S W, Kudrna L and Sakakibara S M. 2006. Effects of clear cutting and tree species composition on the diversity and community composition of epigeous fruit bodies formed by ectomycorrhizal fungi. *Can. J. Bot* 84: 966-980.
- [20] Ray P and Adholeya A. 2008. Development of molecular markers of ectomycorrhizal fungi based on ITS region. *Current Microbiology* 57 (1): 23–26.
- [21] Pena R, Lang C, Naumann A and Polle A. 2014. Ectomycorrhizal identification in environmental samples of tree roots by Fourier transform infrared (FRIR) spectroscopy. *Frontiers in plant science*. 5 (229) 1-9.
- [22] Singh S P, Adhikari B S and Zobel D B. 1994. Biomass, productivity, leaf longevity and forest structure along an altitudinal gradient in the Central Himalaya. *Ecological Monograph*, 64(4): 401-421.
- [23] Bagyaraj D J. 1989. Mycorrhizas, In “Tropical Rain Forest Ecosystem: Biographical and ecological studies” edited by Leith, H. and Werger, M.J.A. Elsevier, Amsterdam-Oxford-New York- Tokyo.
- [24] David Moore, Geoffrey D. Robson & Anthony P.J. Trinci. Published 2011 by Cambridge University Press ISBN: 9780521186957.
- [25] Kavety R. 2007. Influence of Ectomycorrhiza on exudates of *Pinus sylvestris* (L.) *Geophysical Research Abstracts* 9.
- [26] Wipf, D. 2013. International Innovation - Disseminating science, research and technology, Investigating nutrient uptake and exchange in biographic interactions.
- [27] Aggangan N S, Dell B, Malajczuk N. 1998. Effects of chromium and nickel on growth of the ectomycorrhizal fungus *Pisolithus* and formation of ectomycorrhizas on *Eucalyptus urophylla* S. T. Blake. *Geoderma* 84:15-27.
- [28] Beiler K J, Durall, D M, Simard S W, Maxwell S A and Kretzer A M. 2010. Mapping the wood-wide web: mycorrhizal networks link multiple Douglas-fir cohorts. *New Phytologist* 185: 543-553.
- [29] Treeby M, Marschner H and Romheld V. 1989. Mobilization of iron and other micronutrient cations from a calcareous soil by plant-borne, microbial and synthetic metal chelators *Plant and Soil* 114: 217–226.
- [30] Marx D H, Ruehle J L and Cordell C E. 1991. Methods for studying nursery response of trees to specific ectomycorrhiza In *Methods in Microbiology*, pp. 383–411, edited by J R Norris, D J Read, and A K Verma London: Academic Press.