Chapter 7
Ectomycorrhizal Mushrooms: Their Diversity, Ecology and Practical Applications

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Abstract Ectomycorrhizal symbiosis is formed by a large number of plants and fungi. It is an association of fungal mycelia and roots of plants, mostly woody trees. Ectomycorrhiza are formed by fungi like *Russula*, *Lactarius*, *Boletus*, *Cantharellus*, etc. which are mostly common edible mushrooms. The trees which form ectomycorrhiza are *Shorea, Pinus,* etc. Ectomycorrhiza has been proved in at least 162 genera and more than 5400 species. Previous studies were based on morphology of ectomycorrhiza, but molecular data were lacking. However, relatively recently molecular studies and identification have confirmed ectomycorrhiza association of various fungi. These are formed by mostly members of *Basidiomycota* and *Ascomycota*. The orders like *Agaricales*, *Boletales*, *Pezizales*, *Helotiales*, and *Cantharellales* include the largest number of ectomycorrhizal lineages. In tropical regions, trees belonging to *Dipterocarpaceae* and *Caesalpiniaceae* form most ectomycorrhiza. There are attempts to study ectomycorrhiza in India but are way behind the studies that are been conducted around the world. Some of the studies conducted in India are related to ectomycorrhizal mushroom diversity and synthesis but none on genomics, ecological, and physiological studies. This chapter discusses from the basics what are ectomycorrhiza and their ecology and also applied aspects of ectomycorrhiza.

7.1 Introduction

Fungi along with other microbes are essential component of forest and grassland ecosystems because of their role as parasites, causal agent of various infections, decomposers of organic matter, and mutualistic symbionts (lichens and mycorrhizae). Fungi are vital for biodiversity and various ecosystem processes thus balancing the ecological system of earth. Fungi forming conspicuous sporocarps or fruiting bodies (popularly called as mushrooms or toadstools) are mostly either plant parasitic, saprobic, or mycorrhizal. Mycorrhizae are highly evolved,

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A. Varma et al. (eds.), *Mycorrhiza - Function, Diversity, State of the Art*,
DOI 10.1007/978-3-319-53064-2_7

Table 7.1 Types of mycorrhiza and their characteristics

Type of mycorrhiza	Characteristics
Arbuscular mycorrhizas (AM)	Formerly referred to as vesicular-arbuscular mycorrhizas (VAM), they are most prevalent with more than 80% of plants species showing an association involving few fungal genera in the glomeromycota. Most common diagnostic feature is the development of intercellular hyphae, intracellular hyphae and arbuscules in root cortical cells, and production of spores on intra- and extraradical hyphae
Ericoid mycorrhizas	These are found in ericaceae and epacridaceae families of angiosperm. They are important in land ecosystems where soil nitrogen is bound in organic compounds. The nitrogen in these is accessed by plant primarily via fungi associated with fine roots. Epidermal cells of these "hair roots" are colonized by fungal hyphae forming intracellular hyphal bundles or complexes. They belong to ascomycotina and few deuteromycotina
Arbutoid and monotropoid mycorrhizas	These specialized mycorrhizas found in ericales differ structurally from ericoid mycorrhizas in having Hartig net as well as intracellular hyphae and develop a hyphal complex in epidermal cells. The epidermal cells are invaded by a single hypha forming a "peg" around which the host cell elaborates a wall and plasma membrane. Arbutoid mycorrhizas, on the other hand, develop a hyphal complex in epidermal cells
Ectendomycorrhizas	These resemble ectomycorrhiza by having a mantle and Hartig net. However, they are confined to the conifer genera *Pinus* and *Larix* and are formed by a small group of ascomycete fungi
Orchid mycorrhizas	These are restricted to orchidaceae family and are unique in that fungal associations occur with embryo cells of germinating seeds (Peterson et al. 1998) as well as with roots of seedlings and mature plants. In both situations, various fungal species of basidiomycotina form intracellular, short-lived coils called "pelotons," this degenerates and is digested by the host cell

mutualistic associations between soil fungi and plant roots. The symbiotic association is between members of kingdom *Eumycota* (phyla *Basidiomycota*, *Ascomycota*, and *Glomeromycota*) and most vascular plants, especially trees. Fungi form mycorrhizal associations with about 85% of the world's vascular plants (including herbs, shrubs, and trees). Mycorrhizas are generally characterized into seven categories (Table 7.1). Two of the seven types of mycorrhizae are well studied. Endomycorrhizae or Arbuscular Mycorrhizae (AM) is formed by several plants by a limited number of fungal species (ca. 150) in the monophyletic, phylum *Glomeromycota* (Schübler et al. 2001). Ectomycorrhizal (ECM) fungi are much more diverse (>5400 spp.) members of *Basidiomycota* and some *Ascomycota* forming association primarily with woody plants (Read 1991a, b).

Although the term "mycorrhiza" means the association of fungi with roots, relationships called "mycorrhizal associations" are found between hyphal fungi and the organs of higher plants (of different morphological origin) involved in absorption of water and nutrients. In particular, mycorrhizal infection usually

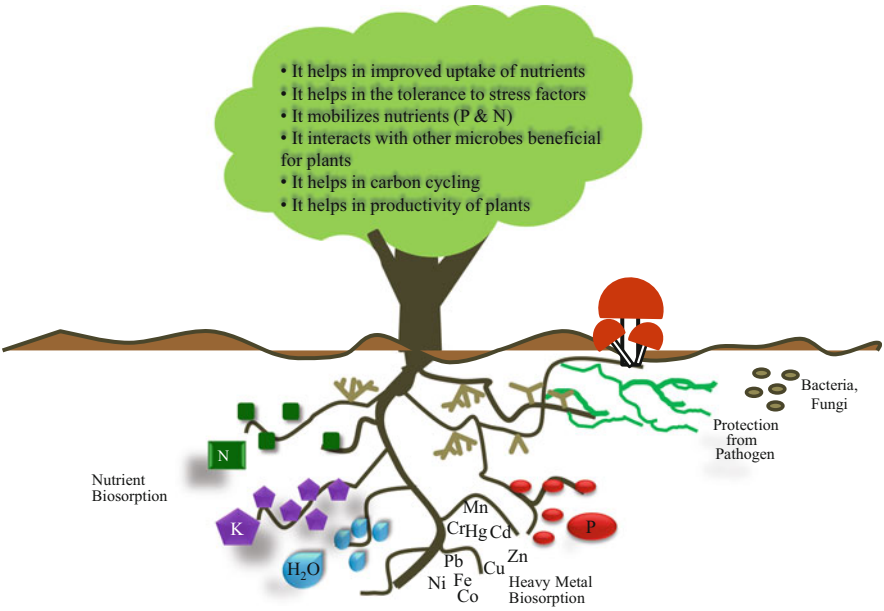

Fig. 7.1 Beneficial effects of ECM fungi

increases the efficiency of nutrient absorption of plants from which the fungus obtains carbon compounds (Fig. 7.1). In mycorrhizas, there is always some penetration of the tissues or a structural modification of roots. Mycorrhizal infected and noninfected roots are clearly distinguishable. The mycorrhizal condition differs from disease as both partners are in normal condition and mutually benefit each other. They are dependent upon one another and interchange of material takes place between their living cells. It was Frank (1885) who recognized and named "mycorrhizen" or "mycorrhiza" for infected roots of temperate forest trees (beech and pine) which are morphologically different from uninfected roots. He later named them as "ectotrophisch" or "ectotrophic" as they possessed conspicuous fungal tissue (sheath or mantle) surrounding plant roots.

Over the past years, research on ECM has shifted from morphological study of ECM to studies on community structure of ectomycorrhizal mushrooms using uncultured approach. Most of the studies focus on the diversity of fungi making ectomycorrhizal association with trees especially those which are edible, understanding the causes and results of an ectomycorrhizal association between plant and fungus, community structure of a forest, biology of ectomycorrhizal mushrooms, synthesis of ECM in laboratory conditions, etc. Recently, Högberg et al. (2001) have studied relative contributions of roots, ECM fungi and free-living microbial heterotrophs to soil respiration using girdling of forest trees.

The present book chapter discusses various aspects of ectomycorrhiza, viz., mycorrhizal categories, morphology of ECM, diversity (plant and fungal), ecology,

artificial synthesis, applied aspects, and recent advances in ectomycorrhizal research.

7.2 Mycorrhizal Categories

Mycorrhizal organs may take different forms depending on the nature of higher plant and fungus, and common kinds have been classified and named. But it is now clear that there is much similarity in general physiology with some detailed specialization between different kinds so that their previous bases of separation have been questioned. The pioneer work of Frank (Smith and Read 1997) resulted in the recognition of two broad subdivisions of mycorrhizas, ecto- and endomycorrhizas. ECM forms Mantle and Hartig net of intercellular hyphae on roots of tree species. AM forms arbuscule, vesicles which are more variable than ECM in that it forms symbiosis with herbaceous plants in addition to tree species (Fig. 7.2). Endomycorrhizas are further classified as arbuscular mycorrhizas, ericoid mycorrhizas, arbutoid mycorrhizas, monotropoid mycorrhizas, ectendomycorrhizas, or orchid mycorrhizas (Table 7.1). Each of these categories is characterized by the invasion of plant root cells by fungal hyphae but differ in the nature of intracellular hyphal development (Peterson et al. 2004). The present chapter discusses various aspects of ECM.

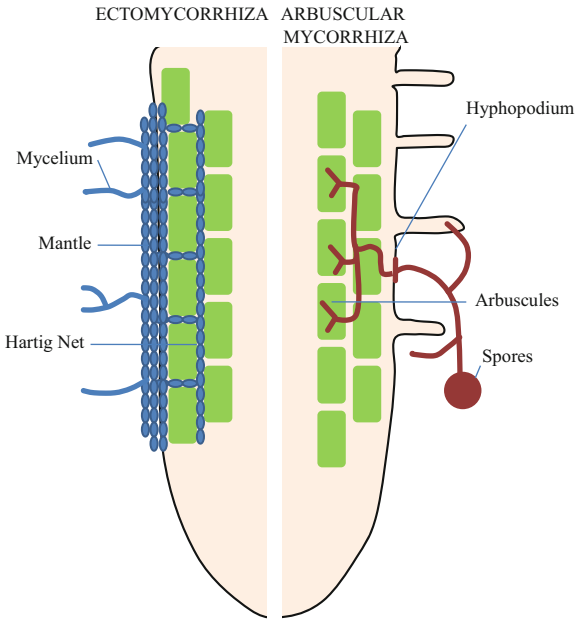

Fig. 7.2 Difference between ectomycorrhiza (ECM) and arbuscular mycorrhiza (AM)

7.3 Ectomycorrhiza

An ECM is a mutualistic symbiotic relationship characterized as a root-fungus association in which the fungus grows on root surface and penetrates the cortex intercellularly to produce a network. ECM mostly occurs in temperate, boreal, and tropical forests (Danell 2002; Dahlberg 2001; Smith and Read 1997; Cairney and Chambers 1999; Verbeken and Buyck 2001; Comandini et al. 2006; Wang and Qiu 2006; Rinaldi et al. 2008). There is a considerable variation in morphological and structural characteristics of ECM. Three features are generally recognized to typify this association:

- Formation of a mantle or sheath of fungal hyphae that covers considerable portions of lateral roots
- Development of hyphae between root cells to form complex branched structure called Hartig net
- Hyphae that come out from the mantle and grow into surrounding soil (extraradical mycelium)

In addition, some ECM develops linear aggregations of hyphae (rhizomorphs and strands) in the extraradical mycelium specialized for rapid transport of nutrients and water. A few ECM fungi develop sclerotia consisting of compact storage hypha surrounded by a ring. Hypogeous or epigeous reproductive bodies are formed periodically from extraradical mycelium. The hyphae do not normally penetrate the cells. Generally, the fungal sheath is usually 20–100 μm thick (often 30–40 μm) and comprises 25–40% of the dry weight of the whole organ. The presence of large fungal component of the absorbing organs suggests that the sheath have selective advantage perhaps in nutrient absorption or storage. This consideration led Lewis (1973) to suggest that the name of ECM be altered to "sheathing mycorrhiza." Hyphal connections run from the sheath between the cells of epidermis and cortex of the plant root, forming the "Hartig Net." Usually there is little hyphal penetration into the cells of plant root of young mycorrhizas, but in senescent parts of a mycorrhizal axis, the cortex becomes colonized by hyphae within the cells.

The presence of Hartig net led some authors to use the term "ectendotrophic" for organs called "ectotrophic." The term "ectendotrophic" (now ectendomycorrhiza) was coined to designate mycorrhizal organs with sheaths/variable development, Hartig net, and extensive intracellular penetration. In addition, considerable research on mycorrhiza of juvenile pines and other conifers especially in nursery conditions has led to the recognition of ectendomycorrhizas, which may merge into the so-called pseudomycorrhizas (believed to be pathological structures) recognized by Melin (1923). There is also considerable variation in the development of various morphological structures. In the extreme, there may be no Hartig net, as in mycorrhiza of *Pisonia grandis* (Ashford and Allaway 1982) or in superficial ECM roots of *Fagus* (Brundrett 2002), or no sheath formation but only a Hartig net as in *Pinus*. ECM associations predominantly forms on fine root tips of host, which are unevenly distributed throughout the soil profile being more abundant in top soil

layers containing humus than in underlying layers of mineral soil. The hyphae of ECM fungi are widely distributed through the soil and make large contribution to nutrient uptake and cycling in ecosystems.

Most of us, as biologists, are familiar with the concept of symbiotic association between ECM mushrooms and vascular plants. ECM biomass can account for up to 25% of the total forest root biomass (Pande et al. 2004). The biological role of ECM mushrooms in ecological niche includes uptake of dissolved mineral nutrition, protection from disease-causing pathogens, balancing the ecosystems, etc. These are achieved by various structures of ECM which also make morphological changes in root.

7.4 Morphology of ECM

ECM has received rather separate consideration from the whole root systems which bear them but more emphasis should be given to the root systems of ECM plants and relatively less on mushrooms forming ECM. Besides the ECM of forest trees, there are variants which depart conspicuously in structure from them like the ectendomycorrhizas. They have reduced sheath of surface hyphae, a well-developed Hartig net and intracellular penetration of living hyphae into living cells. Although we have gained knowledge of root systems of temperate ECM trees, very little is known of those in the tropics, subtropics, or semidesert regions. In temperate forests the active roots of ECM trees are intensely developed in surface and subsurface layers of the soil. Humic layers, especially ECM which form the main component of the feeding system, often lie below the second layer in great quantities. The roots tend to grow in a lateral or upward direction colonizing the newly accumulated humus and litter. In the extreme, smallest short roots of *Pinus* have very small meristematic region, grow little, and differentiate mature stellar and cortical tissues close to the apex. In most of the ECM observed in the forest of *Shorea robusta* (Sal) in Madhya Pradesh, India, the ECM roots were below the thick layer of litter. Sometimes, the fruiting body could be excavated along with attached root of *Shorea* confirming the ECM association between the mushroom species and host plant, *S. robusta* (Figs. 7.3, 7.4). In spite of variation, the common ECM types of most trees are similar in general structure. Besides the presence of fungus on surface of mycorrhiza and between the cortical cells, the lesser development of mature tissues behind the meristem is a conspicuous difference. The features of mycorrhiza are, therefore, those of a slow-growing organ, in contrast to the uninfected root. When growth rates of mycorrhizal roots were compared, they grow slower than uninfected roots. Uninfected roots grow approximately five times as fast as mycorrhizas. Moreover, the roots infected with ECM show more branching than noninfected roots. The profuse root branching also help in the forest to identify the ECM infected roots (Figs. 7.3, 7.4).

In ECM, the modifications of branches of root are of two kinds, roots of potentially unlimited extension in length and roots of restricted growth and viable

Fig. 7.3 (**a**–**b**) Gross morphology of ectomycorrhizae of *Shorea robusta,* dichotomies of short toots. (**b**) Mycorrhizal roots of *Shorea robusta* (sal). (**c**) Ochre brown rough monopodial-pinnate ECM of *Lactarius*. (**d**) Cross section of *Lactarius* ECM. (**e**) Monopodial mycorrhizae showing bulbous and rounded apices covered with hyphal sheath. (**f**) Cross section of ECM of *Russulaceae* member (Sharma 2008)

period. In "heterorhizic" roots, majority of laterals are of limited growth. Apices which are fully infested are usually short and grow slowly, forming racemose branches (Fig. 7.5). Those apices not permanently infested by sheath or uninfected

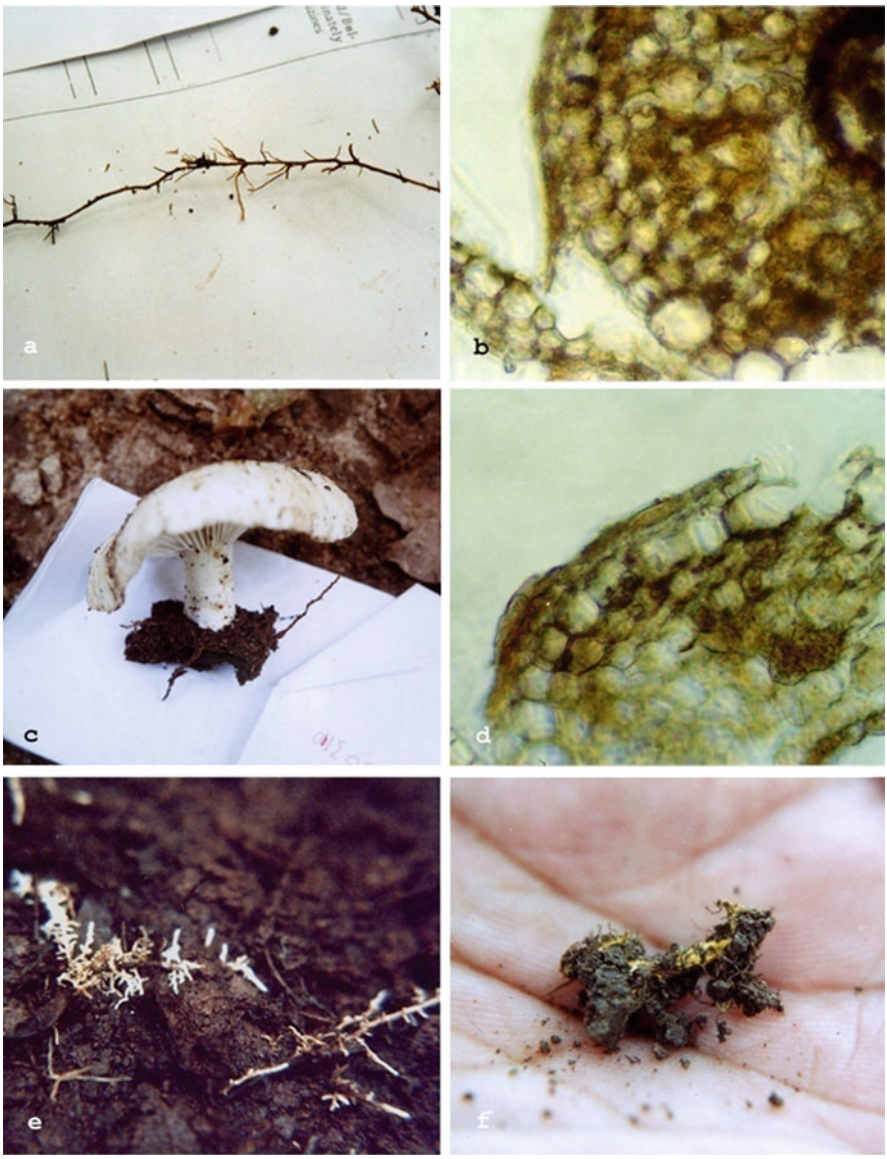

Fig. 7.4 (**a**) Pale yellow monopodial mycorrhizal roots of *Shorea robusta*. (**b**) Cross section of *Russula* ECM. (**c**) *White Lactarius* arising from *Shorea robusta* ECM roots. (**d**) Cross section of *Lactarius* ECM. (**e**) Monopodial mycorrhizae with bulbous and rounded apex. (**f**) ECM of *Pisolithus* with yellow mycelia (Sharma 2008)

either maintain very active growth and are the leading apices of the system or may abort or become dormant (Lakhanpal 2000). *Pinus* departs from this general form because its short roots are sharply differentiated from the axes which bear them.

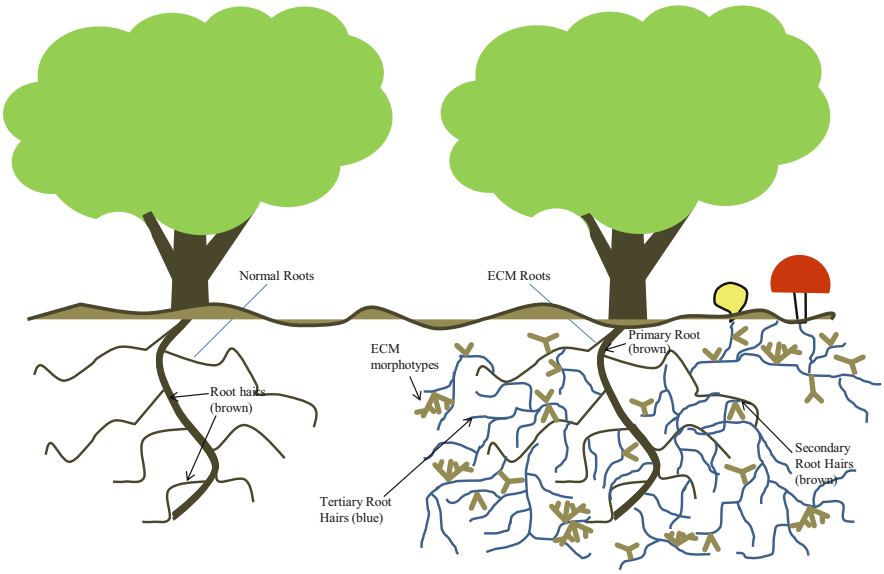

Fig. 7.5 Different types of root morphology in ECM and non-ECM roots

They are simpler in stellar construction, have a restricted apical meristem and root cap, and soon abort if not infected. If infected, they continue to grow, branch dichotomously, and form mycorrhizal systems. "Dichotomy," a characteristic of *Pinus*, is rare or absent in other genera. It is evident that there is an interaction between host and fungi at various levels of ECM development that leads to characteristic features for host-fungus combinations. Agerer (1987, 2002) have described various terms for branching patterns and made into keys. These are also used to identify fungal species associated with host roots. Some common patterns are simple (unramified), monopodial pinnate, monopodial pyramidal, irregularly pinnate, dichotomous, coralloid, and tuberculate. Branching patterns, features of mantle, and extraradical hyphae are used in "morphotyping" field-collected ECM. Tracing hyphal links between sporocarps and roots is a usual way to identify the fungal symbionts associated with roots of particular trees and confirm the symbiosis between both partners. Since the ECM roots and non-ECM roots of a particular tree will differ, it is still followed even in the artificial synthesis experiments. A typical ECM root structure has the following regions: mantle, Hartig net, and extraradical mycelium.

7.4.1 Mantle

The mantle interfaces with root (inner mantle) and soil (outer mantle). It is important in identification of fungal symbionts, interaction with soil microbes,

and its role in movement of water and mineral nutrients from soil solution to root. For identification of ECM fungal component, mantle color and surface features (smooth, warty, cottony or spiny) are also used. Characteristics like the form of outer mantle and presence of cystidia along with patterns formed by interaction of component hyphae (inner, middle, and outer mantle) are used to determine the ECM morphotypes. The mantle of ECM of different trees and/or mushrooms varies in both constitution and thickness. It may appear to be one layer constructed of coherent hyphae or it may be two layered pseudoparenchymatous (Peterson et al. 2008).

With continued hyphal growth, root hairs and old root cap cells are incorporated into the developing mantle and root surface is enveloped by loosely organized hyphae. Some ECM may develop cystidia in the outer mantle. The main tissues of sheath may be reminiscent of those of the sporocarps of ECM fungi. Indeed sheath formed by species of *Tuber* have both active and storage hyphae in them, and those of *Lactarius* contained lactifers. The sheath varies in thickness but is typically 20–40 μm thick and comprises 20–30% of the volume of rootlet. The sheath contribution is estimated to be approximately 39% of dry weight of mycorrhiza.

Although Hartig net is the main crossing point of nutrient exchange in most ECM, the repeated branching of inner mantle hyphae suggests their involvement in the bidirectional movement of nutrients. The ECM fungi are capable of absorbing glucose and/or fructose from root cells and converting them into soluble carbohydrates trehalose and mannitol or into the insoluble carbohydrate glycogen which are stored in mantle hyphae. In addition, mantle hyphae accumulate other compounds including lipids, protein, and/or phenolics and polyphosphates. Deleterious metals may be bound to polyphosphates and other vacuolar deposits in the mantle, thereby preventing their uptake into roots. This feature is significant for tree seedlings inoculated with ECM fungi and planted on metal polluted sites. The compact nature of mantles of ECM protects roots from water loss in dry-soil conditions and from ingress of pathogenic organism through roots. Since the mantle interfaces with the soil, it potentially affects the transport of water and nutrient ions into the root. Bacteria may also be located within hyphae and root cells of some ECM, but the function of these has not been studied much. However, some bacterial species located on the surface of mantle hyphae are known to enhance mycorrhiza formation and consequently plant performance. Perhaps, these make available nutrients to the fungal hyphae.

7.4.2 Hartig Net

The Hartig net comprises of hyphae originating from the inner mantle developed between root cells and forms a complex nutrient crossing point in root. In most angiosperms, the Hartig net develops only around epidermal cells and further development is stopped by thick walls of underlying exodermal cells. One notable exception is the genus *Dryas*, where Hartig net hyphae develop up to the cortex.

Here "phi" thickenings (lignified wall thickenings shaped like the Greek letter Φ) appear to block the ingress of the fungus. In *P. grandis* and perhaps a few other species, a Hartig net is not observed.

In conifer trees, Hartig net develops around both epidermal and cortical cells occupying most of the cortex. During the early stage of Hartig net formation, mechanical intrusion of hyphae into middle lamella between epidermal and cortical cells is evident by the tapered hyphal tips. The process is enhanced by hydrolytic enzymes that soften the middle lamella and adjacent cell walls. It is however widely believed that some degree of pectic hydrolysis of the middle lamella also occurs.

An important feature of Hartig net is its labyrinthine hyphal branching where tubular fungal hyphae are replaced by a multi-digitate mode of growth. It increases the surface area for exchange of nutrients. In some types of ECM like *Fagus* tree, the interface is between unmodified hyphal wall and host cell wall. For *Pinus radiata*, a second type of interface is described as consisting of a modified external layer of host and a modified hyphal wall. Scientists have observed this type of interface in synthesized mycorrhizas of *Betula* with *Amanita muscaria* and in *Pseudotsuga menziesii* and *Betula* in association with ascomycetous fungi (Peterson et al. 2004).

The Hartig net is variously described as uniseriate or multiseriate. The development often depends upon the conditions of culture in artificially produced mycorrhizas. The Hartig net is involved in nutrient exchange, and most of the absorbed sugars, mineral nutrients, and water are passed to root cells through these hyphae. Micro-autoradiographic analysis has shown the movement of sugars from root cells to Hartig net and then to mantle and phosphate movement in the vice versa direction (Bücking and Heyser 2001). Hartig net hyphae also act as a reservoir for carbohydrate, lipids, phenolic compounds, and polyphosphates.

7.4.3 Extraradical Mycelium

Extraradical (extramatrical) mycelia are hyphae that develop from the outer mantle into the surrounding soil. These are extensive network penetrating the soil and interconnecting roots of the same plant and/or adjacent plants. It is known with *Cenococcum* ECM that a certain number of hyphal layers are necessary in the mantle for extraradical mycelium initiation. Soil particles adhere to individual hyphae of ECM fungi or groups of hyphae, colonies of bacteria, excrement of earthworms, pollen, litter, and upper layer of organic debris in soil. *Hysterangium* and *Gautieria*, form "mats" of mycelium that bind soil and fine roots encrusted with calcium oxalate crystals. Rhizomorphs can vary considerably in their morphology, color, and internal structure wherein the large number of individual hyphae interconnects each other (Agerer 2001). In complex rhizomorphs, one or more central hyphae (vessel hyphae) are enlarged and modified septa that allow for rapid movement of water and nutrient minerals.

Relatively few ECM fungal species form sclerotia in the extraradical mycelium. Sclerotia (development and structure) have been studied in details for few species: *Pisolithus tinctorius*, *Hebeloma sacchariolens*, *Cenococcum geophilum*, and *Paxillus involutus*. Other mycorrhizal fungi, including species of *Gyrodon*, *Boletus*, *Austropaxillus*, *Cortinarius*, and *Morchella*, are also known to form these structures. At maturity, each sclerotium usually develops a melanized outer covering (rind) that surrounds a cortex (central area) of compact hyphae and a medulla of loosely organized hyphae. Proteins, lipids, polysaccharides, and polyphosphates are stored in cortical region making them ideal propagules. The formation of reproductive bodies (basidiocarps and ascocarps) involves the localized branching of extraradical hyphae, organization of these hyphae into discrete structures, and differentiation of various regions of the sporocarps.

Mobilization, absorption, and translocation of mineral nutrients and water from the soil substrate to plant roots are the main function of extraradical mycelia. In species with rhizomorphs, connecting fine hyphae may pass dissolved nutrients and water for more rapid translocation through the wide diameter central hyphae (vessel hyphae) to the root. Experiments with radioactive isotopes of phosphorus (P) (^{13}P-labeled orthophosphate) have shown that P can be translocated over distances of more than 40 cm through rhizomorphs to roots of colonized plants and subsequently to the shoot system (Finlay and Read 1986a, b). ECM fungi can obtain P from the mycelium network of a saprotrophic fungus and pass P to the host plant. Carbon (C) compounds are translocated in the reverse direction from the host root to the extraradical mycelium for metabolic and growth processes, to developing sclerotia and their storage reserves, and to sporocarps. Production and final biomass of *Laccaria bicolor* basidiocarps are correlated with the rate of photosynthesis of their host *Pinus strobus*. Experiments with radioisotopes of C in the laboratory have confirmed the movement of C from host to fungus as well as from one plant to another through the extraradical mycelium network (Peterson et al. 2004).

7.5 Ecology of ECM Mushrooms

In the past decade, there has been an increasing awareness between ecologist and mycorrhizalogists that mycorrhizal fungi are an integral part of ecosystems and that their ecological function needs to be understood. It has been shown that mycorrhizal fungi contribute to plant diversity, nutrient cycling, acquisition to nutrient sources, and finally to ecosystem functioning. The survey conducted in the forests of Madhya Pradesh and Chhattisgarh states of India yielded several ECM mushrooms. These formed ECM with *S. robusta, Dendrocalamus strictus* trees. These mushrooms are helping in maintaining pure sal forests and the *Shorea* trees in turn are maintaining the fungal diversity. Although the forest slowly turns into mono-tree forest, the ecosystem is healthy due to the tree-fungal symbiosis. This may be one of the reasons why sal forests are mostly mono-tree forest. Moreover, ECM fungi could well contribute to community and ecosystem responses to global

changes. As the climate is changing, the ECM fungi will help its symbionts to adapt it. Also, based on the effects of symbiosis on plant fitness, population ecology, dynamics, and evolution of many plants are unlikely to be fully understood without considering their fungal symbionts. ECM are active living components of soil population having some properties of roots and some of microorganisms. The ECM mushrooms make the forest a different ecosystem altogether from the dry forest like that of *Tectona grandis*. Understanding the distribution and ecology of ECM fungi is important for the ecosystem studies as well as selection of plants for plantation (Giachini et al. 2000).

7.5.1 Ecophysiology, Ecosystem Effects, and Global Change

It has been estimated that approximately 80% of all land plant species form associations with mycorrhizal fungi (van der Heijden and Sanders 2002). The abundance of mycorrhizal fungi is enormous as plants that form a symbiosis with these mutualistic fungi dominate most ecosystems. Moreover, most tree plants form mycorrhizal symbiosis with multiple fungal species. Majority of plants in the European calcareous grassland, American tall grass prairie, temperate deciduous forests, tropical rain forest, and shrub land of the threatened South African cape region are associated with mycorrhizal fungi. In contrast, plant communities of arctic tundra and alpine regions often contain a lower percentage of mycorrhizal plant species (Harnett and Wilson 1999; Onguene and Kuyper 2001). In tropical forests like that of Central India, this is mostly occupied by pure sal or mixed evergreen or dry deciduous forest. As per our survey and observation, the dry deciduous forests are mostly dominated by *T. grandis* (teak) and evergreen forests are dominated by *S. robusta* (sal). As per our observation, there were no ECM fungi observed with teak, whereas sal forest contains most of the ECM fungi. Survey conducted in the forests of Madhya Pradesh and Chhattisgarh yielded many ECM-forming fungi. That means single tree plant species can harbor multiple fungal species. The large quantity of litter also helped in the survival of the surface mycelia of the ECM fungi. When the litter is removed, the surface is completely covered by mycelia mat.

It is well known that ECM fungi have beneficial effects on plant growth especially when nutrients availability is low, which is mainly attributed to improved plant nutrition (Jakobsen et al. 2002; Simard et al. 2002). The diameter of ECM hyphae is up to 60 times thinner than plant roots which help to form extensive hyphal networks in soil penetrating the soil particles pores. That is why ECM fungi explore effectively for nutrients in comparison to plant roots. The roots of many ECM trees are completely encapsulated by fungal mantle. A number of traits from both plant and mycorrhizal fungi determine fungal impact on plant growth making it a complex interaction (Smith 2000; Smith et al. 2000).

ECM fungi exude several extracellular enzymes that break complex organic substances and have access to organic N and inorganic P that can be transmitted to

their hosts. ECM hyphae are the primary structures that acquire nutrients and are considered as sink of carbon (Jakobsen et al. 2002; Simard et al. 2002). It is estimated that 10–50% of assimilated carbon is translocated to ECM roots (Voke 2012). The influence of global changes such as elevated carbon dioxide, N deposition, ozone, UV radiation, and climate may change mycorrhizal associations (Rillig et al. 2002). Recently, several groups are working on the sequestration of carbon in ECM fungi.

7.5.2 Biodiversity, Plant, and Fungal Communities

One of the major goals in ecology is to search for mechanisms that determine biological diversity (Grime 2001). Apart from the presence of ECM fungi, species composition and diversity of ECM fungal communities also affect plant diversity and productivity (Hart and Klironomos 2002; van der Heijden 2002). Moreover, the influence of ECM on plant diversity depends on plant species composition. ECM diversity might also play a role in seedling establishment of forest trees (Jonsson et al. 2001). Evidence shows that the reverse is also true, that plants affect populations and community composition of fungal symbionts. Bever et al. (2002) have taken a population and community approach to look at how fungi affect plant fitness but at the same time how plant species affect ECM fungal fitness.

Because of differential effects of ECM fungal species on plant growth, it is essential to know which factors determine the diversity and composition of ECM communities (Erland and Taylor 2002). Both abiotic soil factors and biotic factors (such as plant species composition) affect the composition of ECM fungal communities. Enhanced levels of available soil N as caused by atmospheric N deposition change the composition of ECM fungal communities. It also alters levels of root colonization of plants associated with ECM fungi (Rillig et al. 2002). It appears that N deposition and soil acidification reduce ECM diversity. This is often accompanied by a shift in community structure so that dominance by one fungus/few species increases. The negative impact of N deposition on ECM fungal diversity leads to reduced viability of many temperate and boreal forests.

The diversity study of ECM fungi in India is less. They are mostly focused in the Himalayan region. In the Himalayan region, an ECM fungus like *Morchella* is found to form ECM with *Pinus*. *Suillus sibiricus*, an edible fungus, has been found to be associated with plantations of *Pinus wallichiana* in northwestern Himalayan region (Sagar and Lakhanpal 2005). However, in the study conducted in the forests of Central India primarily from the extensive surveyed Madhya Pradesh, we collected 61 species of ECM mushrooms belonging to nine genera (Sharma 2008; Sharma et al. 2008a, b, 2009a, c, 2010b). Most of the mushrooms which belong to *Russula*, *Lactarius*, *Boletus*, *Leccinum*, and *Amanita* were found to form ECM symbiosis with the *Shorea* tree (Fig. 7.6). Some species of *Scleroderma* and *Geastrum* were also found to be ECM with the *Shorea* tree. Apart from *Shorea,* we also observed one species of *Pisolithus* on *Eucalyptus*. Recording of important

Fig. 7.6 Diversity of ECM mushrooms found in forests of *Shorea robusta* forming ECM. (**a–e**) Species of *Russula*. (**f**) Species of *Lactarius*. (**g**, **h**) Species of *Boletus*. (**i**) Species of *Strobilomyces*. (**j–l**) Species of *Amanita* (Sharma et al. 2008a, 2009a, 2010d, e)

characters of ECM mushrooms is important for their identification (Fig. 7.7). A table mentioning all isolates is given in Table 7.2. It is observed that the forests of *Shorea* harbor a lot of ECM fungal diversities. ECM fungal diversity has also been reported on plants of *Dipterocarpaceae* (*Dipterocarpus indicus*, *Kingiodendron pinnatum*, *Humboldtia brunonis*) from the southern part of India (Natarajan et al. 2005b). He reported about 30 ECM fungi from the forests of Uppangala, the dominant one being members of *Russula*. Reddy et al. (2005) also reported a new species of *Pisolithus indicus* associated with *Dipterocarpaceae*. A comparison of three prominent ECM mushroom diversity studies of different regions of India is compiled in Table 7.3.

The members of *Dipterocarpaceae* are not only dominant in Indian evergreen forests but also in other Southeast Asian countries. Watling and Lee (1995) reported about 24 ECM fungi which included agarics, boletes, and earth balls. The dominant forms were members of *Amanita*, *Boletus*, and *Russula*. Similarly, many ectomycorrhizal fungal species associated with dipterocarps have been reported from the Philippines, Thailand, Sri Lanka, and Indonesia (Natarajan et al. 2005b). Recently Thomas et al. (2002) have reported a new genus *Anamika* (*A. indica*) under *Hopea* sp. in Wayanad District, India. Relatively recently, the use of molecular methods to identify the ECM fungi has helped in the authentic characterization of ECM fungi and identification of cryptic species. The species richness values for ECM in oak and conifer forest were 43 and 55, respectively, which were close to a midpoint range for similar other forests studied globally. Taxonomic studies of ECM mushrooms in the forest of Western Himalayas regions have been documented by Lakhanpal (1993, 1996). Similar work has also been undertaken in

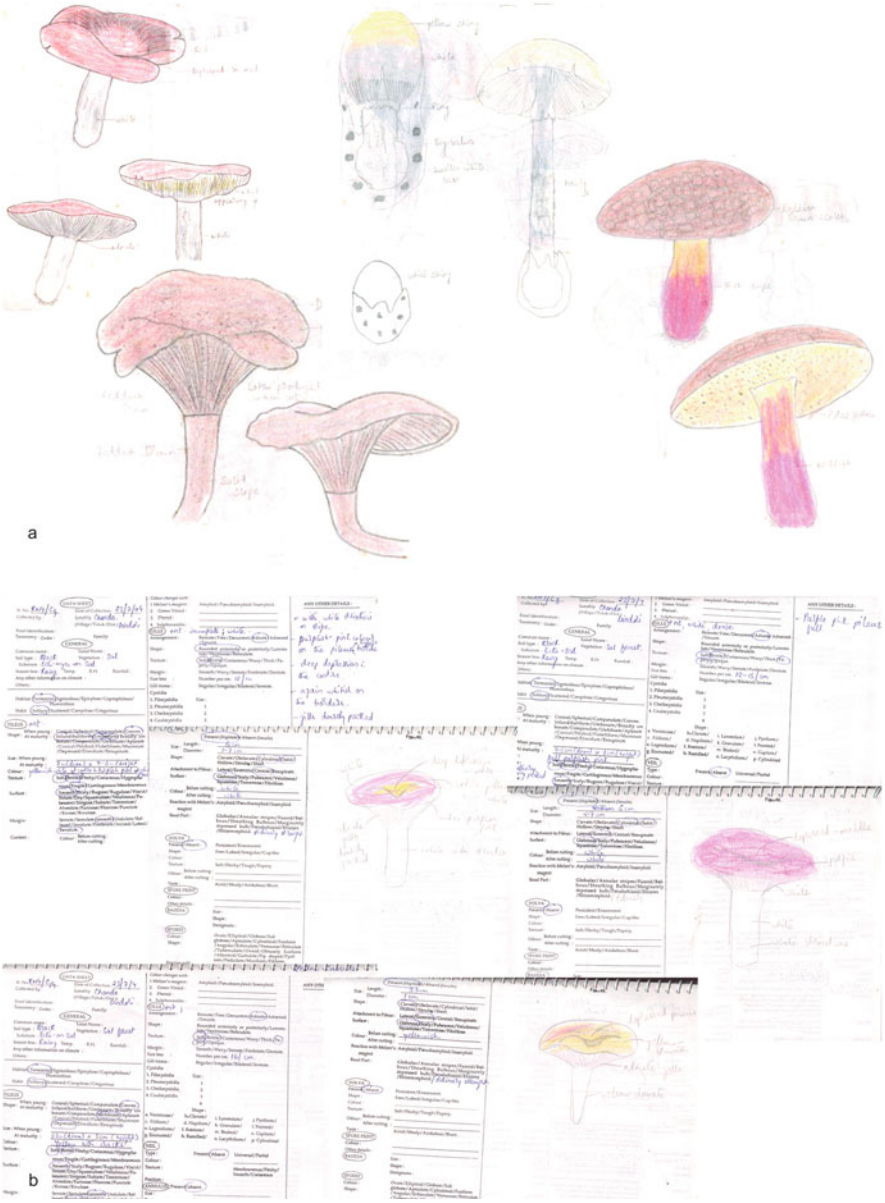

Fig. 7.7 Field notebook helpful in the identification of ECM mushrooms. (**a**) Hand drawings of ECM mushrooms on field. (**b**) Field data sheet filled on field (Sharma 2008)

Western Ghats by Natarajan et al. (2005a, b) and Natarajan and Ravindran (2003a, b). Limitations of estimates include the fragmentary nature of data and consideration of only species associated with dominant trees. Several workers have contributed to the taxonomic and biodiversity status of mushroom (not specifically ECM

Table 7.2 Important genera (in terms of species) of ECM fungi in three India studies (Sharma et al. 2009c)

Genus	Total mycorrhizal species	Percent association with			
		Sal	Teak	Bamboo	Eucalyptus
Amanita	5	+	−	−	−
Russula	24	+	−	−	−
Lactarius	8	+	−	−	−
Boletus	9	+	−	−	−
Leccinum	2	+	−	−	−
Geaster	5	+	−	−	−
Pisolithus	2	−	−	−	+
Scleroderma	5	+	−	−	−
Cantharellus	1	−	−	+	−

mushrooms)—Sharma and Sidhu (1991), Kaul (2002), Sharda (1991), Rattan and Khurana (1978), Khoshoo (1996), Verma et al. (1995), Lakhanpal (1993), and Bhagwat et al. (2000) in the Eastern Himalayan region; Natarajan and Ravindran (2003a, b) and Natarajan et al. (2005a, b) in the Western Ghats of Kerela and Maharashtra; Khoshoo (1991) and Kaushal (1991) in the Northwest Himalaya; Natarajan et al. (2005a, b) in South India; and Saini and Atri (1993) and Purkayastha and Chandra (1976, 1985) in Indo-Gangetic plains (Punjab, Uttar Pradesh and Bengal).

Besides the recognized hotspots in Western Ghats and the North Eastern Himalayan region, Central India is also home to the world's important tropical deciduous forest. These are rich in large unexplored microbial diversity yet to be exploited and conserved. The climatic conditions also make natural habitat for a large number of mushrooms. Central India is home to one of the world's important tropical deciduous rain forests. During the past one decade, regional mycologists launched fungal species richness monitoring studies in central forests dominated by sal, teak, and bamboo, tree species that form the base of region economy. Many studies have concentrated on mushroom diversity but none on ECM mushroom diversity. However, the fact that ECM fungi produce easily surveyed macroscopic sporocarps makes them particularly appropriate for large-scale monitoring studies. In Central - India and nearby regions, the Department of Forestry, Government of India, manages or holds an unusually large number of tracts of young, natural, and ancient teak, sal, and bamboo stands. The presence of variously aged and differently managed stands provided a unique opportunity to compare and contrast fungal communities through time as well as to investigate the impact of timber removal on fungal diversity. In this region, there are few reports on the diversity of ECM mushrooms (Sharma et al. 2008a, 2009a, 2010b, d, e).

Table 7.3 Important genera (in terms of species) of ECM fungi in three India studies (Sharma et al. 2009c)

S. No.	Mushroom species	Western Ghats Natarajan et al. (2005a, b)	Western Ghats Pande et al. (2004)	Central India Sharma et al. (2009a, b, c)
1	*Russula*	12	13	24
2	*Lactarius*	–	9	6
3	*Amanita*	5	15	5
4	*Anamika*	1	–	–
5	*Boletus*	–	12	10
6	*Leccinum*	–	6	2
7	*Strobilomyces*	1	1	1
8	*Suillus*	2	7	–
9	*Cantharellus*	–	–	1
10	*Astrosporina*	3	–	–
11	*Laccaria*	2	1	–
12	*Cortinarius*	1	4	–
13	*Hygrophorus*	–	4	–
14	*Clitocybe*	–	3	–
15	*Hebeloma*	–	2	–
16	*Volvariella*	–	1	–
17	*Inocybe*	–	1	–
18	*Galaria*	–	1	–
19	*Tubaria*	–	1	–
20	*Lacrymaria*	–	1	–
21	*Astraeus*	–	–	–
22	*Scleroderma*	–	–	5
23	*Geaster*	–	–	5
24	*Pisolithus*	1	–	2
25	*Lepista*	–	1	–
26	*Leucopaxillus*	–	1	–
27	*Oudemansiella*	–	1	–
28	*Tricholoma*	–	2	–
29	*Agaricus*	–	1	–

7.5.3 Geographical Distribution and Host Specificity

Despite the long evolutionary history, many mycorrhizal fungi appear to be non-host specific, and available evidence suggests that there is an ongoing parallel evolution of the partners in response to environmental change (Sanders 2002). An interesting phylogenetic analysis suggests that ECM fungi have evolved repeatedly from saprotrophic precursors (Cairney 2000). Unlike AM fungi which are completely dependent on their host for C, many ECM fungi can be cultured separately on agar plates without host roots. Despite their obligate nature, AM fungi are not thought to be host specific.

Plant individuals can be colonized by different species of AM fungi or ECM fungi. Dual colonization by both ECM and AM fungi also occurs in some plant species pointing to the absence of any host specificity. Plant trees show a certain degree of host specificity like only *S. robusta* (sal) forms ECM with mushrooms even in mixed forest where other trees are also present. Unlike AM fungi, not all tree plants form ECM symbiosis. The stability of host specificity is unclear considering that reversals from mycorrhizal mutualists to saprotrophic fungi occur. There is one major exception with regard to specificity in mycorrhizal symbioses, the myco-heterotrophic plants. These plants depend on fungi for the supply of C and photosynthesis is completely or partly lost. Spatial distribution of plants and fungi is often tightly linked due to host specificity. In India, most of the ECM fungi are reported from the Himalayas, Western Ghats, Eastern Himalayas, Central India, and forests of Southern India (Natarajan et al. 2005b; Pande et al. 2004; Sharma et al. 2009c). The same species of mushrooms are found to be forming ECM with different plant tree species in different portions of Earth.

7.5.4 Population and Dynamics

The quantitative measurement of the relations between populations, infection, and plant growth is an important factor to understand responses to efficiency of inoculum and inoculation for adequate ECM formation. Quantitative evaluations in relation to the soil environment are necessary to manage ECM population for maximum effect. The capacity of hyphae to which it can grow from spores, sclerotia, and mycelial strands through soil will affect infection dynamics. Larger propagules like sclerotia and mycelial strands are capable of growth through soil then small propagules (such as basidiospores) for considerable distances obtaining high infection levels.

The longevity of different types of propagules is an important factor for reforestation, but has received little experimental study till now. Basidiospores and sclerotia have considerable longevity, whereas mycelial strands appear fragile and hence have poor inocula in soil. Inoculum potential/infection relations for any one-fungus/plant combination will vary with environmental factors, viz., chemical status, pH, heavy metals, moisture, temperature, organic matter, and micro flora of soil. It is expected that pollutants and acidification of soils can strongly affect ECM formation and fungal spore on roots (Kjøller and Clemmensen 2008). The number of ECM formed under various conditions results from effects of environment on fungal factors in soil and rhizosphere and on root susceptibility to infection and spread.

It is commonly observed that as the forest ages, fungal species which dominated in early stages may be replaced by other fungi and is referred to as “late stage.” The separation of early and late species by no means is clear cut, as some fungal species persist throughout the life of tree plantations, and it is prominent in a situation where an exotic fungal species has been introduced. Moreover, where a natural

situation pertains, as the environment changes with stand age, various fungi will be advantaged and will replace some of the prominent early-stage fungi. Almost all the basidiospores responding to seedling roots are of those species which are regarded as "early-stage" fungi, e.g., species of *Hebeloma, Paxillus*, *Suillus*, *Pisolithus, Scleroderma*, and *Inocybe*. ECM fungi may be associated with several tree species (Sterkenburg 2016). Growth of ECM fungi in rhizospheric soil may be a key factor to understand ECM composition on a root as it ages and to selection of fungi for tolerance of deleterious factors. It has been recognized that studies in laboratory media have little relevance to growth in the rhizosphere, where the physicochemical environment is different. Slow growth of fungi which appear at late stage on young roots as well as poor spore germination may explain their failure to dominate the rhizosphere of young roots. Moreover, some signals (metabolite, chemicals) may be secreted by older tree roots. Fungal communities in general are affected by soil fertility. The development of extraradical hyphae varies considerably between fungus (and hosts) and environment. An understanding of factors affecting growth of extraradical hyphae is important, not only to uptake of nutrient and water but also to other ECM functions. Some ECM fungi, viz., *Russula luteolus*, *Suillus bovinus*, and *Hebeloma crustuliniforme*, form highly branched mycelial strands up to 40 cm (Skinner and Bowen 1974a, b; Finlay and Read 1986a, b).

In recent years, molecular methods have been used to identify the genotypes. Various molecular methods like random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), single-strand conformational polymorphism (SSCP) and inter-retrotransposon amplified polymorphism (IRAP) markers, and repeat SSR markers have helped in knowing the distribution of various genotypes of ECM fungi (Murata et al. 2005; Zhou et al. 2001; Dunham et al. 2003; Kretzer et al. 2004; Wu et al. 2005; Bergemann et al. 2006). They may not completely resolve the genotypes but help in knowing the distribution (Redecker et al. 2001; Dunham et al. 2003; Bagley and Orlovich 2004). The fruiting body collection also helps to know that multiple genotypes of a genus are present in a particular location, like in genera *Russula, Lactarius, Amanita,* etc. Sometimes the spatial distribution is restricted because the distribution of host tree species in a mixed forest influences the distribution of ECM genotypes (Zhou et al. 2000).

7.5.5 Nutrient Circulation in Ecosystem

As a rule, mycorrhizal infection enhances plant growth by increasing nutrient uptake via increasing in the absorbing surface area, by mobilizing sparingly available nutrients sources, or by excretion of chelating compounds or ectoenzymes. Bidirectional transfer of nutrients between plant and fungus is typical of ECM (including other types) and is the basis for the prolonged compatible interactions typical of these symbioses. Depending on the tree species, different amounts of mineral nutrients from the soil to the root occur via fungal hyphae (Marschner

1995). Except where mantle is diffuse, most nutrients delivered to Hartig net are likely to be transported through the living external hyphae. Some ECM have mycelial cords which transport water and nutrients over long distances and in some cases via specialized nonliving hyphae.

At cellular level, interfaces in all types of mycorrhizas are composed of membranes of both partners which are separated by apoplastic region (Smith et al. 1994). The interface is simple intercellular wall to wall contact in ECM, but the fungal partner remains in apoplast space outside the plant protoplast and cell membrane. Fungal colonization of host root tissues is entirely extracellular in ECM fungi. In Hartig net region, the hyphal branch and septa formation profusely becomes uneven or incomplete giving characteristic labyrinthine system (Martin and Nehls 2009). Surface fibrils and acid phosphatase activity present in mantle vanish as hyphae become tightly pressed against host cell walls. Adjacent walls of fungal and host become indistinguishable from each other forming a homogenous interfacial matrix.

They are important for the delivery of carbon to soil and are responsible for a substantial component of forest-soil carbon fluxes (Nehls 2008; Anderson and Cairney 2007; Högberg et al. 2001; Högberg and Högberg 2002; Godbold et al. 2006; Hobbie 2006). However, extracellular material is deposited around hyphae of ECM and also accumulates in intercellular spaces of the fungal sheath in ECM of *Eucalyptus* and *Pisonia* (Pritsch and Garbaye 2011). Evidence (or lack of it) for the nature of the compounds transferred between the symbionts has been reviewed previously by many workers. Sugars are important in carbohydrate transfer with hydrolysis of sucrose (or trehalose in orchid mycorrhizas) along with synthesis of characteristic "nonrecyclable" carbohydrates (e.g., mannitol in ECM), as important steps in polarizing transport in favor of one symbiont (Dearnaley et al. 2012). ECM has a major influence on N and lesser effect on P nutrition wherein inorganic orthophosphate is the major form in which phosphorus is transferred (Wiemken 2007; Baxter and Dighton 2005). Moreover in ECM, the coexistence of ATPase activity on plant and fungal plasma membrane at Hartig net interface suggests that both the systems work cooperatively in bidirectional nutrient exchange. P_i (inorganic phosphorus) transfer to host tissue in excised beech ECM was around 10–20% of P_i absorbed by sheath hyphae, and in mycelia of *P. tinctorius* (Pers.), Coker and Couch net efflux was also 10% of P_i absorbed (Smith et al. 1994).

Hydrolysis of sucrose to hexoses by invertase in the apoplast would allow a net sugar transport to the fungus. The fact that individual ECM roots act as greater sinks for photosynthetically fixed C than non-mycorrhizal roots strongly suggests greater concentration at the interface and the probability of increased loss from the root cells. Release of phytohormones at the interface might also influence nutrient transport. Auxin ,e.g., can increase ATPase activity in plant tissue and may have a role in ECM formation, and the cytokinin $N^{6\text{-}2}$ isopentenyl–adenosine (2iPA) has been shown to influence both absorption and loss of ions from mycelium of the ECM fungus *Suillus variegatus*.

Many studies have shown that ECM fungi can utilize organic N sources through the production of extracellular acid proteinases. Mycorrhizal infection can provide

host plants with access to N sources, which are normally unavailable to non-mycorrhizal roots (Gobert and Plassard 2008). Further, proteolytic capacity may vary greatly between fungal isolates. Possibly all fungi can assimilate ammonia by a combination of the glutamate dehydrogenase and glutamine synthetase pathways, whereas a smaller number of species can efficiently reduce nitrate. Ammonia is rapidly assimilated in the extraradical hyphae and N is transferred to the host primarily as glutamine. ECM symbiosis alters metabolic pathways of N assimilation in the fungal symbiont. Not much is known on the role of mycorrhiza in uptake of K, Ca, Mg, and S. Majority studies on ECM and micronutrient uptake are focused on the protection from excessive uptake of Cu and Zn on soils high in heavy metals. Production of siderophores is widespread between ECM. However, boron is essential for the growth of fungi and ECM may increase concentrations in the host plants. Plant growth or reproduction is not always increased by mycorrhizal infection due to high efficiency of P acquisition or low P requirement of plants. The acquisition of P is reduced when external hyphae are destroyed by grazing soil animals, soil disturbance, or fungicides.

Identification of efficient genotypes of both fungus and host and understanding the way their function is integrated depend on the identification and quantification of the key processes involved in nutrient uptake and use. Despite worldwide research interest, progress is slow in understanding the mechanisms involved, the differences between ECM fungi in their capacity to deliver P to the host plant, and in quantification of the benefit ECM plants have under field and in natural ecosystems. However, better matching of ECM fungi with host and site conditions is required for the full potential of large-scale inoculation programs to be realized. Moreover, recent studies of genome sequencing are giving new insight to the mycorrhizal symbiotic association (Martin et al. 2008).

7.5.6 Interactions with Other Microorganisms

In addition to increasing absorptive surface area of their host plant root systems, the hyphae of ECM fungi provide an increased surface area for interactions with other microorganisms and provide an important pathway for translocation of energy-rich plant assimilates (products of photosynthesis) to the soil (Finlay 2004). These ECM interactions are synergetic, competitive, or antagonistic and have applied significance in areas such as biological control, bioremediation, and sustainable forestry. Bacteria with a potential to fix nitrogen have been discovered which grow symbiotically with tuberculate roots of ECM plants forming rood nodules and also applied as plant growth-promoting rhizobacteria (PGPR) (Cumming et al. 2015). Exudation and reabsorption of fluid droplets at ECM hyphal tips have been earlier demonstrated. The extent to which interactions between ECM mycelia and other microbes affect organic and mineral substrates is unclear, and further experiments are needed to distinguish between the activity of ECM hyphae and activity

facilitated by ECM for the uptake of compounds (Finlay 2008; Churchland and Grayston 2014).

7.5.7 *Multifunctional Role of ECM*

The effects of mycorrhizal fungi have traditionally been considered within narrow perspective of their effects on the mineral nutrition of individual plants. Research during the past 20 years has increasingly viewed symbiotic ECM associations between plants and fungi within a wider, multifunctional perspective. New molecular methods have been applied to investigate ECM fungal communities, and greater attention has been paid to their possible effects at the level of plant community. And we have acquired greater knowledge about fungal species diversity and become more aware of potential functional diversity of ECM fungi. The new multifunctional perspective includes:

> mobilization of N and P from organic polymers; release of nutrients from mineral particles or rock surfaces via weathering; interactions with myco-heterotrophic plants; effects on carbon cycling; mediation of plant responses to stress factors such as soil acidification, toxic metals, drought and plant pathogens; as well as a range of possible interactions with other soil microorganisms.

The role of ECM fungi in shaping terrestrial ecosystems is fundamental. Many of the characteristic plant communities that dominate major terrestrial biomes are due to selection which has favored different types of symbiotic associations that are adapted to soil, vegetation, and climatic conditions characterizing these different environments. Comparative analysis of different systems will improve our understanding of responses to environmental and climatic changes. It is an important prerequisite for future sustainable management of terrestrial ecosystems (Anderson and Cairney 2007; Finlay 2004).

7.6 Practical Applications

7.6.1 *Artificial Synthesis of ECM*

Interest in cultivating edible ECM mushrooms has grown in the past few decades with the realization that there had been dramatic falls in the availability of mushroom species and increased market demand exceeded their supply. Melin (1922, 1923, 1936) developed the pure culture synthesis technique to experimentally demonstrate the ability of known fungus isolates to form ECM with specific hosts under pure culture conditions. Use of these techniques has also led to the discovery of important physiological aspects of symbiosis. These include uptake of nutrients and water by fungus and translocation to the host, movement of photosynthate from

host to fungus, interactions of growth-regulating substances, host-to-host transfer of carbohydrate via a shared fungal symbiont, protection against root pathogens, effects of temperature on mycorrhiza development, specificity and compatibility between fungus and host, and several other processes.

One must recognize the artificiality of pure culture synthesis and limit extrapolation of results to natural situations. Positive synthesis results confirm the ability of that particular host-fungus combination to form ECM. Negative results suggest that union of organisms in question seems unlikely. Melin (1923) primarily used flasks containing sterile sand moistened with a nutrient solution. Several investigators have modified Melin's technique often trying more complex arrangements to reduce the artificial nature of the enclosed system. Hacskaylo (1953) greatly improved the system by using vermiculite instead of sand as the substrate. Vermiculite provides better aeration and moisture holding capacity than sand. Marx and Zak (1965) further improved the substrate by stabilizing the acidity with an addition of finely ground sphagnum peat moss. Molina and Palmer (1982) reports excellent seedling growth and ECM development in the glass test tube system filled with vermiculite and peat moss moistened with nutrient solution which is helpful in running numerous syntheses in a relatively small area. Pachlewski and Pachlewski (1974) also reported good mycorrhiza synthesis in a large test tube but used a solid agar substrate rather than peat moss and vermiculite. Danell (1994) reported artificial ECM formation between *Cantharellus cibarius* with a host tree plant. In another study from India, Sharma et al. (2008b, 2009b, 2010a, c) formed in vitro synthesis of ECM between *D. strictus* and *Cantharellus* sp. (Fig. 7.8). The roots showed root modification and clear interaction between host plant and ECM fungus. The ECM fungal inoculum was produced on used tea leaves and sand (Sharma and Rajak 2011).

7.6.2 Applied Aspects of ECM

Much of our understanding on the functions of ECM has come from research directed toward practical application in forestry. Repeated failures in the establishment of exotic pine plantations in the tropics and other areas where ECM hosts do not naturally occur clearly demonstrated the dependence of these trees on their fungal symbionts. Only after inoculation with forest soil containing ECM fungus propagules could these trees survive and function properly. This information provides many necessary tools and concepts for strengthening forestry programs around the world. Today, wide-scale inoculation of forest nurseries with selected ECM fungi appears imminent. Commercial interest in producing pure culture of ECM fungi inoculum expands the possibilities of worldwide application. The success of these inoculation programs hinges on selection of effective and beneficial fungal symbionts. New inoculation programs must be strongly research oriented from the outset.

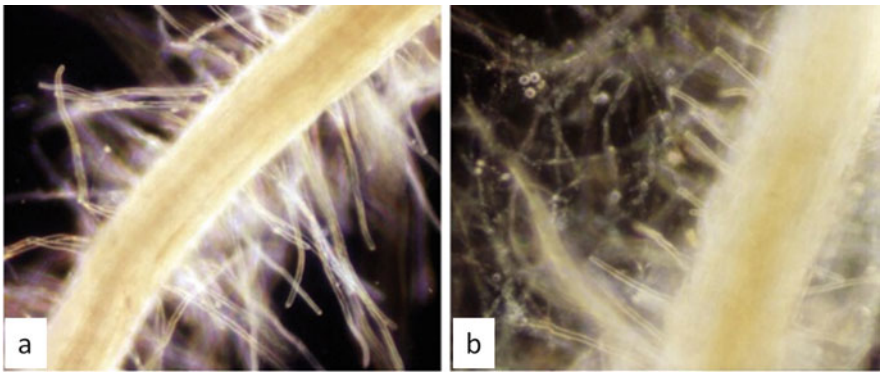

Fig. 7.8 Aseptic ECM synthesis. (**a**) Uninoculated *D. strictus* roots with long root hairs. (**b**) *I*noculated roots of *Dendrocalamus strictus* with *C. tropicalis* with small, less root hairs, and mycelial coverage (Sharma et al. 2009b)

It has been experienced during the introduction of exotic pines into the Southern Hemisphere and Tropical Island that mycorrhizal fungi should accompany host trees. Afforestation attempts in treeless grasslands of the USA and steppes of Russia have also required inoculation for success. Although successful inoculation of tree seedlings (already planted) in field have been known, nursery inoculation is more common. Seedlings inoculated in nursery can establish a healthy ECM system before out planting. Tree seedlings lacking ECM suffer severe nutrient deficiencies early in their first growing season; the deficiencies persist until mycorrhizae are formed. The most commonly used and probably most reliable ECM inoculum is soil taken from beneath ECM hosts. Soil inoculum may also be added to the planting hole when seedlings are out planted. Soil inoculation has been instrumental in the establishment of exotic pine plantations in the Southern Hemisphere and continues as a regular practice there today.

Planting mycorrhizal "nurse" seedlings or incorporating chopped roots of ECM hosts into nursery beds as a source of fungi for neighboring young seedlings has been successful (Sim and Eom 2006). Chevaliar and Grente (1973) were able to inoculate seedlings with the prized truffle fungus *Tuber melanosporum* by use of nurse seedlings already mycorrhizal with this fungus. Basidiospores and ascospores or crushed sporocarps have been used occasionally as inoculum, usually in small experiments. Some investigators have reported good success with this technique. Asexual spores and sclerotia are further sources of inoculum. The gasteromycetes (puffballs and related fungi) with abundant spore masses offer better source of spores than gills. Most of the recent research has been with *P. tinctorius*. Inoculation with spores of *Rhizopogon* species also appears promising. Abundant *Rhizopogon* mycorrhizae formed on seedlings produced from the coated seed of *P. radiata* D. Don with basidiospores of *Rhizopogon luteolus*.

Although many difficulties remain in using pure culture as source, techniques for wide-scale application are now being developed. Unfortunately, many ECM fungi

isolated from either fruiting body or ECM itself grow extremely slowly or not at all in culture. Still, many do grow well in culture, e.g., most species of S*uillus, Hebeloma, Laccaria, Amanita, Rhizopogon*, and *Pisolithus*. Most of the pure culture inoculation has been restricted to small-scale experiments, although Moser (1958) successfully inoculated nursery beds of *Pinus cembra* in Austria with pure cultures of *Suillus plorans* more than 20 years ago. Marx and Bryan (1975) further refined Moser's technique and reported excellent results in inoculating nursery beds with *P. tinctorius*. The logistics of producing massive quantities of inoculum presently limits wide-scale use of pure culture inoculum. Large-scale production methods are now being developed in industrial fermenters or container nurseries by international firms. Other firms are also experimentally producing pure culture inoculum of ECM fungi. Industry representatives and mycorrhiza researchers are optimistic that effective commercial inoculum will soon be available in the market, and it can be used for reforestation of waste and degraded sites (Fig. 7.9).

The promising outlook for pure culture inoculation raises still another important question; which fungus is best for a particular host or habitat? The effectiveness of the various ECM fungi on different host species has to be repeatedly emphasized. With thousands of ECM fungi and numerous hosts, careful selection of the best fungi for particular host is critical. Many important criteria must be considered when selecting fungus candidates for nursery inoculation. Careful experimentation and good record keeping are essential throughout evaluations of each isolate. One must first be able to isolate the particular fungus and grow it reasonably well in culture.

Relatively fast-growing fungi are generally preferred for inoculation because of their short incubation period. Unfortunately, many otherwise desirable ECM fungi grow slowly. According to Marx (1980) fresh cultures are preferred to cultures repeatedly transferred and stored for several years. He further suggests passing important fungus cultures through a host inoculation and mycorrhiza formation followed by re-isolation, every few years to maintain mycorrhiza-forming capacity. Moreover, fungi, which produce large hyphal stands of rhizomorphs in culture of soil, may be superior in soil exploration and mineral uptake to those which lack rhizomorphic growth.

There have been a lot of studies conducted on ECM. In recent times most of the studies are focusing on genome sequencing, genomics, and metagenomics and several other aspects (Martin et al. 2016; Redeker et al. 2004; Tedersoo et al. 2016). There are some interesting articles which have discussed the importance of ECM studies highlighting their role in distribution, evolution, and phylogenetic studies (Tedersoo et al. 2010). Studies are focusing on the one-to-one fungus-plant symbiosis, i.e., between individual partners and benefits shared by them (Kennedy et al. 2015). Some researchers are also focusing on links between community structure and function for ectomycorrhizal fungus (Walker et al. 2014). There is increased to study various aspects of ECM synthesis in detail.

Fig. 7.9 Forest regeneration and reclamation of mine sites. Exploitation of potential ECM mushroom by inoculation of nursery seedlings and tissue culture plantlets of bamboo, sal, and other tree species can help in forest reclamation (Sharma 2008)

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