REVIEW
OF
LITERATURE
2.1. General literature

The term mycorrhiza is derived from Greek word meaning fungus root. This term was coined by Frank to describe the symbiotic association of plant roots and fungi. Stahl (1900) described mycorrhiza as the special manifestation of the soil which is deficient in nutrient content. But Hartig (1886) contradicted this view of symbiotic nature and described this association purely parasitic. Harley and Smith (1983) defined mycorrhiza as association between fungal hyphae and organs of higher plants concerned with absorption of substances from the soil. Kirk et al., (2001) described mycorrhiza as a symbiotic (occasionally weakly pathogenic) association between a fungus and the root of a plant.

A new, broader definition of mycorrhiza that embraces the full diversity of mycorrhiza while excluding all other plant-fungus associations is given by Brundrett (2004): A mycorrhiza is a symbiotic association essential for one or both partners, between a fungus (specialized for life in soils and plants) and a root (or other substrate-contacting organ) of a living plant, that is primarily responsible for nutrient transfer. Mycorrhizas occur in a specialized plant organ where intimate contact results from synchronised plant-fungus development.

The mycorrhizal association and its beneficial role towards plants are accepted as a universal phenomenon (Trappe, 1977). Mycorrhizal associations are so prevalent that the non-mycorrhizal plant is more an exception than the rule (Gerdemann, 1968). The plant groups in which the association does not occur or yet to be reported: the order centrospermaceae and the families cruciferae, cyperaceae, fumariaceae, commelinaceae, urticaceae and polygonaceae (Gerdemann, 1968). However, Gerdemann (1975) has cited exceptions where endomycorrhizae were found for several members of the order centropermae, family chenopodiaceae (Ross and Harper, 1973; Krucklemann, 1975) and several species in the
cyperace and cruciferae (Ross and Harper, 1973). Muthukumar et al., (2004) reported that the family cyperaceae is no longer a non-mycorrhizal family, but the mycorrhizal status of its members is greatly influenced by environmental conditions.

Marschner and Dell (1994) reported that mycorrhizal infection enhances plant growth by increasing nutrient uptake via increase in the absorption surface area by mobilizing sparingly available nutrient sources, or by excretion by chelating compound or ectoenzymes. The mycorrhizal fungi increase the absorption area of the roots and provide host plants with nutrients (N, P, K, Ca, Na, Zn and Cu) resistant to stress and drought and protection against pathogens and pests. As a result of the association, levels of several resistant and inhibitory chemicals (polyphenols and terpenes) increase in plants, beneficial chemicals, including amino acids and hormones, are secreted in high amounts thus increasing the longevity of plants (Dubey et al., 1997). Mycorrhizas are specialized organs formed as a result of the symbiotic association of specific fungi with the feeder roots of higher plants (Swift et al., 2000). The fungus receives photosynthetically derived carbon compounds and the plant has increased access to mineral nutrients and sometimes water (Dell, 2002).

This symbiotic relationship benefits the seedling that develops between themselves and mycorrhizal fungi that occur naturally in any soil environment. This symbiotic relationship improves seedling nutrition in the competitive biological community that inhabits forest soil (Swift et al., 2000). Mycorrhizal fungi increase nutrient and metal acquisition by the plant by strongly increasing the soil volume explored and exploited, by increasing the bioavailability of heavy metals by solubilizing metal bearing minerals and by having high metal uptake capacity (Sarret et al., 2003).

Majority of terrestrial plants form mycorrhiza (Harley, 1989). Over 80% of plant families are mycorrhizal, and this mutualistic
association between plant roots and fungi is the rule in nature, not the exception (Malloch et al., 1980). The arbuscular mycorrhizal fungi are widespread geographically and have a very extensive host range, the ectomycorrhizas are more restricted, forming association predominantly with genera of important woody plants (Dell, 2002). Wang and Qiu (2006) reported that 80-92 % of surveyed land plant species and families are mycorrhizal, whereas arbuscular mycorrhiza is the predominant and ancestral type of mycorrhiza in land plants. The ectomycorrhiza and its derived types independently evolved from arbuscular mycorrhiza many times through parallel evolution.

Initially, the mycorrhizae were of two types ectotrophic and endotrophic (Frank, 1885), later they were renamed as ectomycorrhiza and endomycorrhiza (Peyronel et al., 1969). The five more types have been added to them i.e ectendomycorrhiza, orchidaceous, ericarious, arbutoid and monotropoid mycorrhiza (Harley and Smith, 1983). Since the present investigation exclusively deals with ectomycorrhizae of Picea smithiana and Boletopsis leucomelaena, a review of only ectomycorrhizal literature is given in the following pages.

2.1.1. Ectomycorrhiza

Ectomycorrhizae, the predominant type of mycorrhiza of temperate forest ecosystems, are characterized by a well-developed fungal mantle or sheath surrounding the plant root, and a network of hyphae (the Hartig-net) which penetrates between, but not inside, root cells (Trofymow et al., 2000). It is a specialized root organ, which is the result of a complex interaction leading to a finely-tuned symbiosis between a plant and a compatible ectomycorrhizal fungus (Harley and Smith, 1983).

About 10% of world floras are colonized by ectomycorrhizae, especially in trees belonging to the Pinaceae (pine, larch, spruce, and hemlock), Fagaceae (oak, chestnut, beech), Betulaceae (alder, birch), Salicaceae (poplar, willow), Juglandaceae (hickory, pecan), Myrtaceae (Eucalyptus), Ericaceae (Arbutus), and a few other form
ectomycorrhizae. Some tree genera such as Alnus, Eucalyptus, Casuarina, Cupressus, Juniperus, Tilia, Ulmus and Arbutus form both ectomycorrhizae and vesicular arbuscular mycorrhizae (VAM), depending on soil conditions and tree age (Harley, 1969; Hacskaylo, 1971; Marks and Kozlowski, 1973; Harley and Smith, 1983; Molina, 1994). Ectomycorrhizal association comprises the feeder roots of most commercially important conifers (Wiensczyk et al., 2002).

Over 5000 species of fungi all over the world can form ectomycorrhizae on some 2000 species of woody plants. Among the basidiomycetous fungi, species of Hymenomycetes (Mushrooms) in the genera Boletus, Cortinarius, Suillus, Russula, Gomphidius, Hebeloma, Tricholoma, Laccaria, Lactarius and species of the Gasteromycetes (puffballs) in the genera Rhizopogon, Scleroderma, and Pisolithus form ectomycorrhiza. Certain orders in the Ascomycetes such as Eurotiales (Cenococcum geophilum), Tuberales (Truffles) and Pezizales have species that form ectomycorrhizae on trees. Many ectomycorrhizal fungi can be grown routinely in pure culture. They can not exist saprophytically in nature without a plant-host association, spores or resistant hyphae may survive long periods in soil without a plant host, but the fungi from these propagules will not grow independent of their plant host as saprophytes (Trappe, 1962; Kendrick and Berch, 1985). Ectomycorrhizae of conifers are formed by hundred of species of fungi, mostly Basidiomycota and Ascomycota and include host of the fungi that form large mushrooms in forests (Trofymow et al., 2000).

The spores or hyphae (propagules) of the fungal symbionts inhabiting the rhizosphere of the feeder roots initiate ectomycorrhizal infection. Propagules are stimulated by root exudates and grow vegetatively over the feeder (short) root surface, thus forming a fungal mantle (Harley and Smith, 1983). Ectomycorrhizae consist of a fungal sheath surrounding plant roots and a Hartig net that penetrates into the intercellular space of plant cortical cells (Satomura et al., 2006).
The "Hartig net", may completely replace the middle lamella between the cortex cells. It is the main distinguishing feature of ectomycorrhizae (Smith, 1974; Harley and Smith, 1983). Ectomycorrhizal fungi colonize roots, modify root colour, shape and function and often are characterized by extensive external hyphal development (Amaranthus, 1998).

Ectomycorrhizae may be unforked, bifurcate, nodular, multiforked (Coralloid), or in other shapes. Their colour, which is usually determined by the colour of the mycelium of the fungal symbiont, may be black, red, yellow, brown, white, or blends of these colours. Colonization by the ectomycorrhizae is limited to the primary cortex (with intercellular hyphae surrounding cortical cells) and does not spread beyond the endodermis or into meristem tissues of the feeder root (Martin and Hilbert, 1991).

There are much larger, physiologically active root fungus areas for nutrient and water absorption with trees having abundant ectomycorrhizae than trees with few or no ectomycorrhizae. This increase in surface area comes both from the multi-branching habit of most ectomycorrhizae and from the extensive vegetative growth of hyphae of the fungal symbionts from the ectomycorrhizae into the soil. The extrametrical hyphae function as additional nutrient and water absorbing entities and assure maximum nutrient capture from the soil by the host. Ectomycorrhizal roots are able to accumulate nitrogen, phosphorus, potassium, and calcium in the fungus mantle more rapidly, and for longer periods of time than the non-mycorrhizal feeder roots (Marx and Shafer, 1989).

Ectomycorrhizal mycelia differ in their density, organization and extension as well as in their biomass. As these mycelia are very important for nutrient uptake, they act as sink for carbohydrate and other minerals (Agerer and Raidl, 2004).

Ectomycorrhizal roots have greater longevity (length of physiological activity) than non-mycorrhizal roots (Slankis, 1973). The
associations of ectomycorrhizal fungi with tree roots enhance the acquisition of phosphorus from the soil. In addition to increase the uptake of $\text{H}_2\text{PO}_4^-$ ($\text{P}_i$), mycorrhizal fungi may increase the spectrum of P sources utilized by tree roots by mediating the dissolution of insoluble metallophosphate salts or the hydrolysis of organic P compounds (Cumming, 1993).

Ectomycorrhizal fungi are crucial to many ecosystem functions and have great ecological and economic values. These fungi are essential in many forest food webs (Amaranthus, 1998). Ectomycorrhizal inoculation procedure has been developed to improve forestation practice in areas with unfavorable habitat (Bratek et al., 2002).

2.1.2. The fungal sheath or mantle:

Detailed description of fine structure of the mantle was given by Foster and Marks (1966) and Scannerini (1968). The fungal mantle consists of weft of interwoven hyphae enveloping short roots. Hyphae in the mantle may be loose or tightly interwoven. Different ectomycorrhizal fungi with different hosts form distinctive mantles in varying thickness, texture and colour. The hyphal sheath is formed by plectenchymatous hyphae (Dominik, 1959; Chilvers, 1968a; Godbout and Fortin, 1985). The characteristics of hyphal mantle can best be recognized in tangential sections (Haug et al., 1986).

Mantle may be smooth or with radiating hyphae which enter into soil to increase the absorbing surface of short roots. Mantle surface can range from thin to profuse and texture can vary from smooth, cottony, velvety and warty to granular (Zak, 1973). The hyphae radiating from mantle surface may be simple or branched bearing simple or clamped septa. Colour of these hyphae may be hyaline, black or in various shades such as orange, yellow and brown. The colour of mantle is mainly due to the colour of the radiating hyphae (Marks and Foster, 1973).
The fungal hyphae in the mantle are more or less coated with a cement which has been described as a coating layer (Scannerini, 1968), an interfacial matrix (Scannerini and Bonfante-Fasolo, 1983), and apposition layer (Duddridge, 1986a, b; Nylund, 1981; Strullu, 1974) or the external layer of the hyphal wall (Strullu, 1986). In mycorrhizas formed by Ascomycetes, the cement is electron dense, whereas it is transparent in Basidiomycete mycorrhizas (Scannerini and Bonfante-Fasolo, 1983). However, for some authors, this property depends more on the stage of mycorrhizal development than on the type of fungal symbiont (Kottke and Oberwinkler, 1986). Various studies have attempted to determine the nature and origin of the cement. It appears to consist of polysaccharides (Duddridge, 1986a, b; Massicottle et al., 1986) associated with proteins (Dexheimer and Gerard, 1988).

Ectomycorrhizal colonization is limited to the primary cortex (with intercellular hyphae surrounding cortical cells) and does not spread beyond the endodermis or into merismatic tissues of the feeder roots (Martin and Hilbert, 1991). Bucking et al. (2002), studied the apoplastic permeability of the fungal sheath of two (Pisolithus tinctorius and Suillus bovinus) different ectomycorrhizal associates of Pinus sylvestris.

Gross et al. (2004) studied the ectomycorrhiza formed between Pinus caribaea with Pisolithus tinctorius and Thelephora terrestris. The results showed that ectomycorrhizal fungi formed well developed compact mantle of closely packed hyphae and covered the mycorrhizal roots. Agueda et al. (2006) described the ectomycorrhizae formed by the Boletus edulis and found that the mantle was plectenchymatous and the hyphae were lacking clamp connections.
2.1.3. Hartig net:

The region where host and fungus come into close contact where nutrient exchange takes place between the two symbionts is known as “Hartig net”. Hyphae from the fungal mantle also penetrate through the epidermis into the intercellular spaces of the cortical cells apparently replacing the middle lamellae and form an interconnecting network i.e Hartig net. In most cases, this network spreads slowly inwards until it reaches the endodermis, which effectively bars any further penetration, though in some angiosperms the hartig net may not penetrate beyond the first layer of cortical cells. The fungus and root cell retain their vital characteristics and show no disease symptoms (Marks and Foster, 1973).

The penetration of hyphae between the cells of the root cortex does not lead to plasmolysis or other deleterious cytological alterations in the host cells. As the hyphae insinuate themselves between the cortical cells, the latter simply separate at the middle lamellae, and an almost complete single layer of fungal hyphae eventually separates and virtually encapsulates each cell, though plasmodesmata connections may remain between cortical cells (Marks and Foster, 1973). The presence of fungal net actually prolongs the life of the cortical cells and of the root as a whole (Kottke and Oberwinkler, 1986a, b; Massicotte et al., 1986).

It has been shown that sugars are translocated from the root via the Hartig net to the fungal mantle, where they tend to accumulate (Strullu, 1976; Nylund, 1981). As sugars pass from plant to fungus, they are converted into trehalose (a diasaccharide), mannitol (a polyhedric alcohol) and glycogen, all three being typical fungal carbohydrates. The glycogen is insoluble, and therefore unavailable for possible reabsorption by the plant (Strullu, 1976; Kottke and Oberwinkler, 1986a).

Rayner (1927) described that the Hartig net is hyphal in nature and made up of septate hyphae. Strullu (1976) described the Hartig
net as consisting of ‘lames fungique’. Electron microscope examination (Atkinson, 1975; Duddridge, 1980; and Nylund, 1981) of Hartig net reveals that it consists of complicated fan-like systems of hyphae which provide a very large surface of contact between cells of the two symbionts. With regard to septation, it has been opined that the walls of hyphae in close contact have been erroneously interpreted as septate or incompletely septate (Hofsten, 1969; Marks and Foster, 1973). The double wall nature of the structures reveals, however, that they are apparently not septate and that incomplete septae are the beginning of the branches (Duddridge and Read, 1984; Kottke and Oberwinkler, 1986b).

The process of Hartig net development has been reviewed by Kottke and Oberwinkler (1986b). It starts when hyphae come into contact with unsuberized living cortical or epidermal cells and is characterized by changes of hyphal growth and morphology. The diameter of the hyphae may be greater (Kottke and Oberwinkler, 1986a; Massicotte et al., 1986) than that of hyphal sheath. Hyphae are oriented transversely to the root axis and began to branch out irregularly, septation is rare, the hyphae penetrate in the direction of the endodermis and grow in longitudinal direction through the intercellular spaces is rather restricted.

Dell et al., (1990) studied the ectomycorrhizal roots of Eucalyptus and showed that these roots have Hartig nets penetrating to the hypodermis and are similar to the superficial eucalypt ectomycorrhizas formed in soil and litter. Agueda et al., (2006) described the ectomycorrhizae formed by the Boletus edulis and reported the lobed and septate structure of Hartig net.

2.1.4. Characterization and identification of ectomycorrhizae:

Since the investigations by Melin (1922, 1923, 1925), many synthesis of mycorrhizae using mycelia from known fruit-bodies have been reported. In Melin’s (1923) opinion identification of mycorrhizae by discovering hyphal connections with fruitbodies is too uncertain
because of the difficulties involved in following the hyphae between these structures. Since the early twenties numerous attempts have been made to identify mycorrhizal fungi.

Melin (1927) as the first to classify ectomycorrhizae of pines into four groups on the basis of gross-morphology. Dominik (1956) followed a more detailed system, incorporating many more categories and utilizing anatomical features. Both methods have been stated to be too general and both fail to give satisfactory identity to mycorrhizae. These methods, however, gave a direction to characterization and identification of ectomycorrhizae and later attempts were limited to single tree species defining distinct, individual natural mycorrhiza. Fontana (1962) described and illustrated 16 different ectomycorrhizae on roots of 14 species and varieties of *Salix*. Marx (1975) characterized seven mycorrhizae on *Pinus radiata*. Rambelli (1966) described and illustrated mycorrhizae on roots of nursery seedlings of the same species.

Fungal symbionts, however, were not identified in either of the studies. Chivers (1968a) characterized eight ectomycorrhizae on *Eucalyptus* and also gave indentities of two fungal symbionts.

Ceruti and Bussetti (1962) identified fungal symbionts of five ectomycorrhizae of *Tilia* species and Fassi and De-Veccehi (1963) provided names of fungi of four of the five described mycorrhizae of *Pinus strobes* nursery seedlings. Fontana and Centrella (1967) named symbiotic fungi of eight mycorrhizae of *Castanea, Carpinia, Fagus,* *Pinus* and *Quercus* species. Trappe (1965) described a black mycorrhiza and a tuberculate mycorrhiza of Douglas fir. He also described (1967) four Douglass-fir mycorrhizae synthesized in pure culture of *Hebeloma crustuliniforme,* (Bull. Ex St. Amans) Quel., *Suillus subolivaceous* Smith and Thiers, *Rhizopogon colossus* A.H. Smith and *Astraeus pteridis* (Shear) Zeller.

Zak (1969) described two new ectotrophic mycorrhizae of Douglas fir each of which is formed by a distinct strain of *Poria*
terrestris (DC. ex Fries) Sacc. He named each mycorrhiza according to tree and fungal species including strain of the letter as denoted by characteristic staining of respective sporocarps. Zak (1971) laid down certain criteria for characterization and identification of mycorrhizae. Zak (Ic) characterized and classified ectomycorrhizae formed by Rhizopogon vinicolor with Pseudotsuga menziesii roots. He re-examined the tuberculate mycorrhiza of Douglas fir and opined that the fungal symbiont described earlier as a phycomycete and basidiomycete by Trappe (1965), was actually Rhizopogon vinicolor. Zak (1971) also described the cultural characteristics of this fungus.

One more method sometimes used to identify mycorrhiza (Ceruti and Bussetti, 1962; Chu-chou and Grace, 1981, 1982, 1983a; Pachlewski, 1968; Thomas and Jackson, 1979; Zak and Larsen, 1978) involving the comparison of culture obtained from mycorrhizal roots with cultures of known mycorrhizal fungi from the same plots. This method however, may lead to errors because different fungi can have very similar characteristics when grown in pure culture. On the other hand strain specific differences of one and the same species may exist and thus falsely suggest different species.

Ectomycorrhizae formed by Lactarius rufus and Picea sitchensis in the field and under aseptic conditions are described and compared (Alexander, 1981). Acsai and Largent (1983) described fifteen ectomycorrhizae for Abies concolor of which the mycobiont is known for six. They also described thirteen ectomycorrhizae for Pseudotsega menziesii, of which three mycobionts were identified. Agerer (1986a) described comprehensively the methods applied for characterization and identification of ectomycorrhizae and also summarized the available literature. Agerer (1986b) described and identified the mycorrhizae of spruce with Lactarius deterrimus, L. picinus, Russula ochrolenca and R. xerampelina by tracing hyphal connections between the fruiting bodies and the mycorrhizae.
Ectomycorrhizae of *Cortinarius obtusus* and *C. venetus* on spruce are comprehensively described and compared by Agerer (1987a). They differ in some anatomical, morphological and chemical features and in autofluorescence. Agerer (1987b, c) studied the mycorrhizae formed by *Dermocybe. Cinnamomea, D. sanguinea, Trichoderma sulfureum* and *T. vaccinum* on spruce. Treu (1987) characterized, identified and compared the ectomycorrhizae formed by *Suillus aeruginascens* on *Larix decidua*is. Agerer and Weiss (1989) characterized, identified and compared the naturally grown and synthesized ectomycorrhizae formed by *Thelephora terrestris* on *Picea abies*.

Agerer (1992) described the ectomycorrhizae of *Boletopsis leucomelaena* in detail. The most important features are thick emanating hyphae, which are inflated at their clamps, and occasionally have small amyloid surface structures, and chlamydospores formed within oidia-like cells. Pera and Alvarez (1995) studied the ability to form ectomycorrhiza with *Pinus pinaster* in pure culture synthesis of 98 isolates of putative mycorrhizal fungi, mainly collected in Northern Spain. A total of 35 species in 16 genera—*Amanita, Cenococcum, Collybia, Cortinarius, Hebeloma, Laccaria, Lactarius, Lyophyllum, Melanogaster, Paxillus, Pisolithus, Rhizopogon, Scleroderma, Suillus, Thelephora* and *Xerocomus* formed ectomycorrhizae. Many of these species were not previously reported as symbiotic with *Pinus pinaster*. Hashimoto and Hyakumachi (1998) determined the distributions of ectomycorrhizas and ectomycorrhizal fungal inoculum with soil depth (0-45cm) in a 40 year old *Betula platyphylla var. japonica* forest. The ectomycorrhizas were mainly distributed (>50%) in the top soil (0-5cm) of organic forest floor horizons.

Matsuda (1999) identified and described the ectomycorrhizal roots formed by *Strobilomyces confuses* associated with *Abies firma*. Eberhardt et al., (2000) identified and characterized the
ectomycorrhizae formed by *Lactarius subsericatus*, *L. intermedius* and *L. salmonicolor* on silver fir. Wurzburger *et al.*, (2001) characterized and described sixteen ectomycorrhizal morphotypes, nine each from the mixed conifer and hydric pygmy communities. One morphotype (Black) was found on every site and two morphotype were found on both hydric pygmies (white and yellow).

Jackus *et al.*, (2005) documented and characterized ten tomentelloid ectomycorrhizae isolates collected from *Populus alba*, *Quercus cerri* and *Picea abies*. The investigated ectomycorrhizae belong to the brown-black tomentelloid morphotype but form two different anatomotype groups (At I and At II). Agueda *et al.*, (2006) characterized and described the ectomycorrhizae formed between the *Boletus edulis* and *Cistus ladanifer*. They described that the ectomycorrhizae had traits typical of Boletales: whitish with three differentiated plectenchymatous layers in the mantle in plain view forming ring-like structures and rhizomorphs with highly differentiated hyphae.

Lehto *et al.*, (2008) described that tomentella formed black-brown, brown, yellow, or ochre ectomycorrhizae on the roots of gymnosperms and angiosperms trees, distinguished by typical morphological-anatomical characteristics (clamped hyphae, angular mantle, surface network, special rhizomorphs and cystidia).

### 2.1.5. Systematics of fungal symbionts:

Many species of fungi are normally involved in the ectomycorrhizal associations of a forest stand, on a single tree species, on an individual tree, or even on a small segment of lateral root. As many as three species of fungi have been isolated from an individual ectomycorrhiza (Zak and Marx, 1964). A single fungal species can enter into ectomycorrhizal association with numerous tree species of the same site. A fungus can also develop numerous biotypes or clones in a very limited area of a pure stand (Fries, 1987). Some fungi are apparently host specific; others have broad host ranges and form
ectomycorrhizas with members of numerous tree genera in diverse families (Marx and Cordell, 1988).

An estimated 5000 fungi can establish ectotrophic mycorrhizae with about 2000 woody hosts. All ectomycorrhizal fungi, with only a few exceptions, belong to Basidiomycetes and only some to Ascomycetes (Trappe, 1962). Miller (1982) recorded mycorrhiza forming activity in representatives of 73 Basidiomycete genera distributed among 27 families in nine orders.

Most ectomycorrhizal fungi can be accurately identified only from their macroscopic fruit bodies, which are produced during a relatively short season each year. General technical works for the identification of ectomycorrhizal fungi are those of Singer (1975), Moser (1978), Ainsworth et al., (1973 a, b). Good illustrations can be found by Cetto (1978, 1979 a, b).

Many species of Boletaceae have been shown to be ectomycorrhizal, and most are believed to be so (Singer, 1965; Trappe, 1962; Miller, 1982). Sharma (1980); Sharma (1986) and Watling and Gregory (1980) have collected and described species of *Suillus* (in N.W. India) with *Pinus wallichiana*. Sharma and Lakhanpal (1981) recorded 20 species of boletes which form mycorrhizal associations with forest trees like *Pinus wallichiana*, *Quercus* sp., *Rhododendron* sp. and *Betula* species.

The Asian *Suillus* species (Miller and Lee, 1987) and North American species (Palm and Stewart, 1984) have been confirmed to be ectomycorrhizal by synthesis experiments and European species (Treu, 1987) confirmed by direct observations of hyphal connections between basidiocarps and ectomycorrhizae.

Cotter (1987) while working on the systematics ecology of boletes with special reference to the genus *Suillus* and its ectomycorrhizal relationships, recognize and described nine species of *Suillus* from Nepal. Provided synoptic keys to the basidiocarps and to
cultures of different species worked out by him. Mycorrhizal synthesis confirmed that the six Suillus-Pinus relationships are ectomycorrhizal.

Cazares et al., (1992) reported 24 species of hypogeous fungi from Mexico, one being Rhizopogon guzamanii sp. nov. and 17 being first reports from Mexico. Hintz (1993) reported 54 taxa of hypogeous fungi found in Germany. Mora and Garza (1997) collected 493 specimens of mushrooms from Mexico (including Boletopsis leucomelaena), they belong to 186 species and 107 genera, 6 species were recorded for the first time.

Ozturk et al., (2003) collected macrofungi specimens from Alanya district in the Mediterranean region (including Boletopsis leucomelaena). They worked out 188 taxa belonging to two classes and 38 families. Eleven taxa belonged to Ascomycetes and 177 to Basidiomycetes. Kasik et al., (2003) reported 94 taxa of macrofungi (including B. leucomelaena) belonging to 28 families from Turkey. Out of these specimens 85 belonged to Basidiomycotina and 9 belonged to Ascomycotina. In a similar study Turkodlu and Gezer (2006) recorded 62 macrofungi belonging to Basidiomycetes and 7 belonging to Ascomycetes.

2.1.6. Physiological studies on mycobionts:

France and Reid (1984) tested four ectomycorrhizal fungi for their ability to grow (i.e, mycelial mat radial extension and fungal biomass) on nutrient media either supplemented with ammonium-nitrogen or nitrate-nitrogen or in the absence of an inorganic nitrogen source. Pisolithus tinctorius, Cenococcum geophilum and Thelephora terrestris exhibited greater growth on ammonium-nitrogen. Suillus granulatus grew better on the nitrate-nitrogen nutrient medium. Rapoir and Andary (1987) found that Cortinarius orellani mycelium grew to 5 cm in diameter in 28 days.

The effects of temperature, pH, salt tolerance and water stress on the growth of Boletus griseus and Suillus grevillei by growing them
on Potato dextrose agar and modified Melin-Norkran's media were investigated by Peng and Chien (1988). They selected suitable strains and optimum growth condition (for reproduction of ectomycorrhizal inoculum) on the basis of these studies. Chang and Chien (1988) cultured species of ectomycorrhizal fungi on MMN medium in a temperature range of 5 to 40°C. They concluded that optimum temperature for most of the strains ranged from 22 to 25°C with the optimum at 25°C.

Erland et al., (1990) measured the growth rates of ectomycorrhizal fungi isolated from Pinus sylvestris in pure culture at pH 3-8 on different liquid and solid media.

Blaudez et al., (2000) tested 39 ectomycorrhizal isolates of Paxillus involutus, Pisolithus tinctorius, Suillus bovinus, S. luteus and S. variegates on Cd, Cu, Ni and Zn amended media to determine their in vitro tolerance. S. luteus, S. variegates and Pisolithus tinctorius were more tolerant to Cu, Cd and Zn when compared with Paxillus involutus whereas the reverse was true for Ni.

Castro et al., (2002) grew three strains of Cantharellus cibarius on liquid media containing ammonium, nitrate and bovine serum albumin in different combinations and found that most readily utilisable source of nitrogen was ammonium. Niemi et al., (2002) found that both the mycelium and culture filtrate of Pisolithus tinctorius contained more free and conjugated IAA than the mycelium and culture filtrate of Paxillus involutus after a 3-week time.

Yamanaka (2003) studied that ectomycorrhizal species showed optimum growth at pH 5 - 6. Hatakeyama and Ohmasa (2004) studied the effect of glucose and ammonium tartrate concentrations on nine strains of the genera Suillus and Boletinus on Ohta medium. The result indicated that these fungi were adapted to relatively high concentration of carbon sources.
2.1.7. In vitro synthesis of ectomycorrhiza:

*In vitro* mycorrhizal synthesis studies provide the direct and scientifically rigorous means of determining ability of a fungal isolate to form mycorrhizae. Morphology and anatomy combined with other distinctive characters provide essential data for identifying mycobionts from field connections (Palm and Stewart, 1984; Godbout and Fortin, 1985).

Melin (1921, 1922, and 1936) successfully demonstrated for the first time that ectotrophic mycorrhizae could be produced in synthetic cultures by inoculating seedlings of *Picea abies*, *Pinus sylvestris* and *Larix europaea* with appropriate fungi. Thereafter, he and other investigators have applied this basic technique for studies in determining the identity of mycorrhizal fungi and in physiological experiments dealing with mycorrhizae. The most used and most successful substrate for synthesis cultures has been sand moistened with a nutrient solution.

Marx and Zak (1965) further improved the substrate by stabilizing the acidity with an addition of finely ground sphagnum peat moss. Marx and Bryan (1970) synthesized ectomycorrhizae by *Thelephora terrestris* and *Pisolithus tinctorius* on different conifer hosts. They observed that mycorrhizae formed by *T. terrestris* were macroscopically and microscopically different from those of *P. tinctorius*, but mycorrhizae formed by different isolates of *T. terrestris* were indistinguishable from each other, regardless of host. Results suggested that the fungal symbiont determines colour and morphology of ectomycorrhizae.

Marx and Ross (1970) synthesized ectomycorrhizae on *Pinus taeda* by basidiospores of *Thelephora terrestris*. Cultures isolated from the mycorrhizae were identical with the culture used to form the basidiocarp. Zak (1976) described pure culture synthesis of *Arctostaphylos uvaursi* mycorrhizae with *Laccaria laccata*, *Lactarius sanquifluus* and *Pisolithus tinctorius*. Zak (1976) observed close
resemblance between morphologies of natural and synthesized ectomycorrhizae of Douglas fir, western hemlock (*Tsuga heterophylla*) and ponderosa pine (*Pinus ponderosa*), formed by the same fungi.

Molina (1979) tested cultures of 208 ectomycorrhizal fungi in pure culture synthesis for mycorrhiza formation with red alder. Only four of the 28 fungi tested formed characteristic ectomycorrhizae. Fortin et al., (1980) described synthesis of ectomycorrhiza on *Pinus strobes* seedlings within five days after inoculation with *Pisolithus tinctorius*. They also described techniques for the observation of early morphological changes during ectomycorrhizae formation.

Duddridge et al., (1980) synthesized mycorrhizal rhizomorphs with aseptically germinated seedlings of *Pinus sylvestris* and *Suillus bovines* mycelium, and studied their role in water transport. Tozzi et al., (1981) described the mycorrhizal synthesis between *Boletus edulis* and *Quercus pubescens* by inoculation of mycelium from malt agar, on seedlings growing in vermiculite. Alexander (1981) described and compared ectomycorrhizas formed by *Lactarius rubus* and *Picea sitchensis* in the field and under aseptic conditions. Nylund and Unestam (1982) studied the process of *in vitro* mycorrhiza formation in Norway spruce using the fungus *Piloderma croceum*. They discussed a hypothetical model for host fungus interactions regulating the mycorrhizal infection process.

Palm and Stewart (1984) reported mycorrhizal synthesis between *Pinus resinosa* and *Suillus americanus*, *S. brevipes*, *S. luteus*, and *Suillus neoalbidipes* and between *Pinus strobes* and *Suillus americanus*, *S. brevipes*, *S. granulatus*, *S. pictus* and *Suillus punctipes*. Mycorrhizae formed by all combinations were similar in that all had multiple dichotomous branches and in mantle organization. Mycorrhizae differed in the macroscopic colour of the mantle and surrounding hyphae and hyphal strands.

Warmbrodt and Eschrich (1985) studied ectomycorrhiza of *Pinus sylvestris*, synthesized *in vitro* with *Suillus variegatus*. They
compared the structure of mycorrhizas produced in vitro, with that to naturally occurring mycorrhizas on the same host species. Ceruti et al., (1986) worked out the mycorrhizal synthesis between Boletus aereus and Castanea sativa by inoculation of mycelium from culture, in vitro on seedlings in sterile vermiculite. Miller et al., (1986) achieved mycorrhizal synthesis under laboratory conditions between Amanita muscaria and Pinus taeda and Pinus virginiana. They reisolated the fungus from the mantles and verified its identity by cultural characteristics.

Brunner (1993) applied various in vitro synthesis techniques to produce numerous ectomycorrhizae between *Picea abies* and *Hebeloma crustuliniforme* seedlings. Danell (1994) found that automatic addition of a diluted mineral solution supplemented with glucose and a filtered air flow with 0.2% carbon dioxide was essential for *Cantharellus cibarius* growth and mycorrhiza formation. Successful mycorrhiza formation was repeatedly observed after 8-12 weeks. Cripps and Miller (1995) studied the ectomycorrhiza formed in synthesis tubes by *Populus tremuloides* seedlings and each of seven fungal isolates.

Feugey et al., (1999) inoculated the roots of *Betula pendula* with the *Paxillus involutus* and *Hebeloma cylindrosporum*. These fungi showed different rates of mycorrhiza formation in vitro. Mature mycorrhiza was obtained after only 2-4 days with *Hebeloma cylindrosporum*, whereas 6-8 days were necessary for *Paxillus involutus*.

The first in vitro aseptic synthesis of mycorrhiza between *Abies firma* and *Pisolitlus tinctorius* and *Cenococcum geophilum* has been reported by Vaario et al., (1999; 2000). Langer et al., (2008) found that the inclusion of micronutrient and vitamins in a MMN-based medium increases survival rate to 60% and supported successful mycorrhizal synthesis between *Populus tremula* and *Paxillus involutus*.

### 2.1.8. Sources of inoculum and inoculation techniques:

Ectomycorrhizae may be initiated by several different kinds of inoculum, which can be categorized as: natural inoculum in the form of air borne spores; soil already colonized by an ectomycorrhizal fungus or fungi; seedlings already colonized by an ectomycorrhizal fungus or fungi, that is bearing mycorrhizal roots; fungus sporomata, spores or sclerotia specially collected for the purpose; and fungal mycelium produced in axenic culture. Each has advantages and disadvantages in relation to the objectives and economics of the inoculation programme. If the procedures are followed properly, these
methods usually produced abundant ectomycorrhizae on seedlings (Bowen, 1965; Mikola, 1973; Trappe, 1977, Marx, 1980).

Soil inocula taken from beneath ectomycorrhizal host tree have been used extensively, especially in developing countries (Mikola, 1970). In bare root nurseries, up to 10% by volume of soil inoculum is incorporated into the soil (top 10 cm of beds) before sowing. Parke et al., (1983) reported enhanced growth of Douglas fir container seedlings inoculated with litter and humus taken from beneath Douglas fir trees. One of the most serious disadvantages of soil inoculum is that weed seeds, rhizomes and potential pathogen may also be transported into the nursery with the soil.

Spores or macerated fruiting bodies of some ectomycorrhizal mushrooms, puffballs, or truffles provide good inoculum. Spore inoculum is prepared by blending freshly collected fruit bodies with tap water at high speed for two to three minutes. Li and Castellano (1987) and Li (1987) have found beneficial microorganisms within and on the surface of mature fruiting bodies of various ectomycorrhizal fungi; these organisms should be encouraged, not excluded (Garbaye and Bowen, 1987; Linderman, 1988). Spores are applied 6 to 12 weeks after sowing, either with a standard watering can or through the existing irrigation system. Spores can be applied to the seed before sowing (Marx and Bell, 1985; Marx et al., 1984; Theodorou, 1984; Theodorou and Bowen, 1973). Spores of different fungi have been successfully used to inoculate and stimulate growth of pines in Australia (Theodorou, 1971; Theodorou and Bowen, 1970, 1973) and South Africa (Donald, 1975). Marx (1976, 1980) and Ruehle (1980) have had similar success with inoculating *Pisolithus tinctorius* onto assorted pine species in the United States. However, one of the major disadvantages of spore inoculum of most ectomycorrhizal fungi is the lack of appropriate laboratory tests to determine spore viability. Another disadvantage is that sufficient sporophores of many fungi may not be available every year.
Pure mycelial or vegetative inoculum of ectomycorrhizal fungi has been repeatedly recommended as most biologically sound material for inoculation (Bowen, 1965; Marx; 1977a, Mikola, 1973; Shemakhaneva, 1962; Trappe, 1977). Molina and Palmer (1982) described isolation and maintenance of ectomycorrhizal pure cultures. Basically, a pure culture of a particular fungus is obtained by isolating fungal material (vegetative tissue explant) onto special media that is then grown under aseptic conditions to produce inoculum (Molina and Palmer, 1982).

Industrial fermentation and entrapment in calcium alginate beads have been successfully employed to produce pure culture inoculum in France (Le Tacon et al., 1988). In Canada, vegetative inoculum of several ectomycorrhizal fungus species has been successfully produced in industrial fermenter for operational applications in container and bare root nurseries (Marx et al., 1991). Mycorrhizal tablets are now commercially available in the Philippines from the National Institute of Biotechnology and Applied Microbiology (Marx et al., 1991).

Moser (1958 a, b, c) in Austria was one of the first to make a serious attempt to produce vegetative inoculum of ectomycorrhizal fungi. For production of inoculum, mycelium of *Suillus plorans* was first grown in liquid culture then in sterile peat moss. Takacs (1961, 1964 and 1967) in Argentina modified Moser’s technique to produce inoculum for new pine nurseries established in formerly treeless areas lacking native ectomycorrhizal fungi. Inoculum of *Amanita verna*, *Suillus granulatus*, *S. luteus*, *Scleroderma verrucosum*, and *S. vulgare* were produced with this technique. Hacskaylo and Vozzo (1967) initiated a series of inoculation experiments with pure vegetative cultures of various fungi. Following Moser’s general technique, they grew *Cenococcum geophilum*, *Suillus cothuranatus*, *Corticium bicolor* and *Rhizopogon roseolus* in polypropylene cups containing a 2:1 ratio of sterile peat moss and vermiculite moistened with nutrient solution.
Marx (1980) discussed in detail the early testing and development stages of producing viable inoculum of *P. tinctorius* for research purpose. Vermiculite based inoculum has been used successfully to form *P. tinctorius* ectomycorrhizae in fumigated soil on pine, oak and pecon seedlings in experimental microplots. (Marx and Bryan, 1975; Marx, 1979 a,b) and on pine seedlings in conventional bare root nurseries in Georgia, Florida, and North Carolina (Marx *et al*.,1976), Virginia (Marx and Artman, 1978) and Mississippi (Marx, 1980). In most of these nursery tests, seedlings with abundant *P. tinctorius* ectomycorrhizae grew larger than control seedlings with naturally occurring mycorrhizae.

Vermiculite based inoculum also has been used successfully in forming *P. tinctorius* ectomycorrhizae on container grown tree seedlings in various media and container types (Marx and Barnet, 1974; Marx, 1975; Ruehle and Marx, 1977; Molina, 1979; Dixon *et al*., 1979; Maronek and Hendrix, 1980; Pawuk *et al*., 1980; Ruehle *et al*., 1981). These authors found that fertility, type of container, growing medium, fungicides, inoculum storage, and frequency of watering all influence effectiveness of the inoculum. Dramatic improvements in survival and growth of pine seedlings with abundant *P. tinctorius* ectomycorrhizae over naturally infected control seedlings produced in containers or bare root nurseries have been reported from studies on acid coal spoils in Appalachia (Marx, 1977a, Marx and Artman, 1979; Walker *et al*., 1980), Kaolin spils in Georgia (Marx,1977a), severely eroded sites of the copper basin in Tennessee (Berry and Marx, 1978), borrow pits in South Carolina (Ruehle, 1980) and North Carolina (Goodwin, 1980) and Prairie soil (Baer and Otta, 1981). Similar improvements in pine seedlings performance were reported on routine reforestation sites by several workers (Marx *et al*., 1977, Mexal, 1980; Ruehle *et al*., 1981).

Alberto and Sevastianos (2002) established a technique to study the ectomycorrhizal fungi on agar medium. Petri dishes, 60mm in
diameter, containing 10ml of culture medium covered with a cellophane disk were used for easy collection of the mycelium after growth. Inoculation was achieved by placing a mycelial block on to the center of the cellophane sheet and then incubating at 25°C in the dark.

Oliveira et al., (2006) studied the viability and infectivity of an ectomycorrhizal inoculum of *Rhizopogon nigrescens* produced by submerged cultivation in an airlift bioreactor and immobilized in beads of calcium alginate gel. The inoculum produced by submerged cultivation displayed a high survival rate under refrigeration, with 100% viability after 18 months. Which indicate a high potential for the commercial application of the inoculum in forest nurseries.

Rincon et al., (2007) studied the vegetative inoculum of *Amanita ovoidea* and three isolates of *Suillus collinitus*. Their results emphasised the importance of selecting compatible fungal-host species combinations for nursery inoculation and sources of inoculum adapted to the environmental conditions of the transplantation site.

### 2.1.9. Selection of the mycobiont:

The most important first step in any nursery inoculation programme is the selection of the fungi (Bowen, 1965; Marx, 1977a; Mikola, 1973; Moser, 1973; Trappe, 1977). One criterion is host specificity. The consistent association of certain fungi for only a few specific tree hosts is well documented in the literature (Trappe 1962). Many other fungi are associated with a great number of different tree hosts (Marx, 1977b; Stevens, 1974; Trappe, 1977). It is imperative, therefore, that the candidate fungi exhibit the physiological capacity to form mycorrhizae on the desired hosts. Another criterion is the ability of the selected fungi to grow in pure culture; many ectomycorrhizal fungi will not. A variety of culture media (Moser, 1958b, Stevens, 1974) and methods of isolation (Palmer, 1971) can be used to obtain pure cultures of the selected fungi. Ideally the fungi should be able to
grow rapidly (Moser, 1959). Takacs (1967) recommends subculturing the stock cultures of the fungi every 60 days in order to retain vigour.

Another criterion is the ecological adaptation of the selected fungus to the major type of site on which the seedlings are to be planted. The candidate fungi must be an early stage fungus in normal succession if it is to be effective on seedlings in the nursery and in the early successional stage of establishment (Moser, 1959; Trappe, 1962). There appear to be distinct early stage and late stage fungi in ectomycorrhizal fungus succession in forests (Marx et al., 1991). Only the early stage fungi are able to rapidly colonize seedlings in natural, non-sterile soil that harbors competitors and other environmental stresses. The effect of temperature on different species of ectomycorrhizal fungi is perhaps the most widely researched environmental factor. Upper and lower temperature limits of the candidate fungi should be determined. Moser (1958a) studied the ability of fungi to survive long periods of freezing at 12°C and to grow at 0 to 5°C. He found that high elevation ecotypes of Suillus variegatus survived freezing for two months. Pisolithus tinctorius can grow at temperatures as high as 40 to 42°C (Hung and Chein, 1978) and has a hyphal thermal death point of 45°C.

Reactions of candidate fungi to soil moisture, organic matter and pH are also important traits to consider. Cenococcum geophilum is not only drought tolerant but forms ectomycorrhizae in natural soils ranging in pH from 3, 4 to 7.5 (Trappe, 1977) Suillus bovines (Levisohn, 1956) and Paxillus involutus (Lahio, 1970 form abundant ectomycorrhizae on seedlings in nurseries with high organic matter and moderate soil moisture. Bowen (1973) showed that nutrient uptake is greater in fungi that produce hyphal strands. The production of sclerotia and hyphal strands by P. tinctorius (Marx et al., 1982) and C. geophilum (Trappe, 1969) in soil enhance the ability of these fungi to survive harsh soil conditions. Therefore, hyphal strands and sclerotia production are also a favourable trend in candidate
fungi. All the criteria mentioned are meaningless unless the candidate fungus is aggressive and can form abundant ectomycorrhizas on seedlings as soon as short roots are produced. The fungus should be able to maintain superiority over naturally occurring fungi on seedlings roots in the nursery (Marx and Cordell, 1988).

Kropacek and Cudlin (1989) suggested that the growth of seedlings was affected positively by the mycorrhiza introduced in the form of granulated inoculum; strain *Laccaria laccata* was found to be an optimal symbiont for *Picea abies* and *Pinus silvestris*, particularly under an increased level of inoculum.

Erland and Soderstrom (1991) studied five ectomycorrhizal fungi in relation to the effects of application of lime and wood ash to the growth substrate and found that *Paxillus involutus* was the only fungus affected both by the pH increase, and by the different treatment applied in all aspects of its ecology tested (growth and survival in humus, infection potential and competitive ability).

Lilleskov and Bruns (2003) while studying colonization behaviour of *Rhizopogon occidentalis* and *Tomentalla sublilacina*, reported that colonization potential of a fungus depends upon resource availability and competitive ability. Hoang and Tuan (2008) have listed similar criterion for determining the colonization potential of different ectomycorrhizal fungi.

### 2.1.10. Artificial inoculation, growth and development of seedlings:

The need of many species of forest trees for ectomycorrhizal association was initially observed when attempts to establish plantations of exotic pines routinely failed until the essential fungi were introduced (Briscoe, 1959; Clements, 1941; Gibson, 1963; Hatch, 1936; Kessel, 1927; Madu, 1967; Van Suchtelen, 1962). The need of pine and oak seedlings for ectomycorrhizae has also been convincingly demonstrated in the afforestation of former treeless
areas, such as the grasslands of Russia and Great Plains of the United States (Goss, 1960; Hatch, 1937; McComb, 1938; Rosendahl and Wilde 1942; Shemakhanova, 1962; White, 1941).

The concept of improving field performance of tree seedlings by forming ectomycorrhizae on them in nurseries with specific fungi ecologically adapted to the planting site was originally developed by Moser (1958) in Austria. Using various modifications of Moser's technique and philosophy, Takacs (1967) in Argentina, Theodorou and Bowen (1970) in Australia, and Vozzo and Hacskaylo (1971) in the United States showed experimentally that field survival and growth of tree seedlings with specific ectomycorrhizae exceeded the performance of seedlings that lacked or had few native ectomycorrhizae at planting.

Moser (1958a, b) has shown that mycorrhiza seedlings artificially inoculated with pure cultures of *Suillus plorans* have a better survival and initial growth after planting than naturally inoculated seedlings. In Australia, artificial inoculation of *Pinus cembra* has now reached the stage of practical application. Few experiments have been conducted on the artificial inoculation of scots pine (*Pinus sylvestris*). Compared to natural inoculum, inoculation with pure cultures of *Laccaria laccata* and *Hebeloma cylindrosporum* improved seedling growth in a bare root nursery (Le Tacon and Bouchard, 1986). Large scale inoculation experiments have been done in the U.S.A. with *Pisolithus tinctorius* on different pine species including *Pinus taeda*, *P. virginiana*, *P. ponderosa*, *Pinus strobes* and *Pinus resinosa* (Marx et al., 1982; 1984).

Marx and Schenck (1983) reviewed the potential of mycorrhizal symbiosis in agricultural and forest productivity. Inoculation of containerized seedlings with pure culture has been practiced by many workers (Dixon et al., 1979; Maronek and Hendrix, 1979; Marx and Barnett, 1974; Marx, et al., 1982; Molina, 1980; Pawuk et al., 1980; and Shaw and Molina, 1980). Molina (1982) succeeded in inoculating
containerized Douglas-fir seedlings with four isolates of *Laccaria laccata*. Thomas and Jackson (1983) inoculated containerized *Picea sitchensis* seedlings with *Laccaria laccata* and obtained well developed mycorrhizas. However, in both cases, the inoculated seedlings growth was not improved over non-inoculated seedlings. Manson and Wilson (1984) have reported improved growth of out-planted Sitka spruce seedlings preinoculated with *Laccaria laccata* or *Paxillus involutus*. Marx and Cordell (1989) described the use of specific ectomycorrhizas to improve artificial forestation practices. Effective mycorrhizal fungus inoculum along with the necessary equipment and technology for successful operational applications in bare root and container nurseries is now available to nursery personal (Cordell et al., 1991).

Cordell et al., (1991) compiled a list of outplanting performance of seedlings inoculated with ectomycorrhizal fungi from the world literature. Sixty six species of ectomycorrhizal fungi have been used experimentally to form ectomycorrhizae on 49 tree species. Over 40% of the publications dealt with *Pisolithus tinctorius* on 29 different tree species. *Cenococcum geophilum*, *Hebeloma crustuliniforme*, *Laccaria bicolor*, *L. laccata* *Suillus granulatus*, *S. luteus*; and *Thelephora terrestris* have been evaluated to a lesser extent on six or more tree species. Marx et al., (1991) produced *Pinus pinaster* seedlings with *Suillus granulatus* ectomycorrhizae and outplanted them to produce edible fruit bodies of the fungus.

Cumming (1993) reported that seedlings shoot and root weights of both nonmycorrhizal and mycorrhizal seedlings increased with increasing P concentration in the nutrient solution. Brandes et al., (1998) reported that mycorrhizas were well established on plants inoculated with *Paxillus involutus*. The percentage of mycorrhizal root tips ranged from 60% in the treatment, mycorrhiza was absent on non-inoculated plants.

Jentschke et al., (2001) reported that mycorrhizas were well established on plants inoculated with *Paxillus involutus*. The
percentage of mycorrhizal root tips ranged from 86% in the treatment without P to 93% in the treatment with P. Kayama et al., (2005) performed experiments of artificial inoculation into different conifers and reported considerable improvement in the growth and development of these seedlings over the control.

Hilszczanska (2005) studied the effect on quantity and quality of ectomycorrhizas after inoculation of Pinus sylvestris with three ectomycorrhizal basidiomycetes: Suillus luteus, Boletus pinicola and Hygrophorus olivaceoalbus. He reported Boletus pinicola as the best fungus.

Yasushi and Ippei (2006) reported the positive role of ectomycorrhizal fungi in the growth and development of coniferous seedlings. Rincon et al., (2007) inoculated seedlings of Pinus halepensis with mycobionts like Suillus collinitus, Amanita ovoidea and Rhizopogon roseolus and reported significant improvement in growth and development of tailored seedlings.

2.1.11. Effect of artificial inoculation on nutrient uptake of seedlings:

Ectomycorrhizal fungi’s beneficial effects on plant nutrition have been known for some time (Melin, 1925; Hatch, 1937; Bowen, 1973). Hatch (1937) reported that mycorrhizal white pine (Pinus strobes) weighed significantly more and contained more nitrogen (N), phosphorus (P), and potassium (K) than did non-mycorrhizal plants. Hatch (lc) demonstrated that in the same substrate plants with mycorrhizae absorbed 234 percent more phosphorus than plants without mycorrhizae; also, 75 percent more potassium and 86 percent more nitrogen were absorbed by the mycorrhizal plants.

It was shown in New Zealand and, Australia that the stimulating effect on conifer growth depends largely on the uptake of phosphorus liberated by the mycorrhizal fungi in the soil (Morrison, 1962; Bowen, 1962, 1965, 1966; Bowen and Rovira, 1961; Hacskaylo
and Vazzo, 1967). The role of the mycorrhizal fungi in the liberation of nutrients from complex compounds in the forest soil has been very much discussed (Melin, 1925, Hatch, 1937; Bjorkman et al., 1967). The inverse relationship between soil fertility and ectomycorrhizal infection has long been recognized (Hatch, 1937; Bjorkman, 1942) and ectomycorrhizal infection is often reduced by application of fertilizers (Richards and Wilson, 1963).

Harley (1969, 1970) has discussed the results of a number of studies of macronutrient uptake by ectomycorrhizal plants. In general, ectomycorrhizae have been found to increase uptake of N, P, and K, but few other elements have been investigated. Ectomycorrhizae increased the Ca content of Pinus radiata (Henderson and Stone, 1970) but other investigators found that neither the Ca nor Mg content of ectomycorrhizal Pseudotsuga taxifolia (Trappe and Strand, 1969), or Pinus caribaea (Hart et al., 1980) was increased.

Bowen (1973) has pointed out that the uptake of trace elements by ectomycorrhizal trees has received little attention. In one of the few studies on minor element uptake, excised mycorrhizal roots of Pinus radiata absorbed more zinc than non-mycorrhizal roots (Bowen et al., 1974).

In many studies involving mycorrhiza, the mycorrhizal association usually increased the growth of plants by enhancing the uptake of nutrients especially phosphorus (Harley and Smith, 1983; Kucey et al., 1989). Mycorrhizal fungi in association with plant roots seem likely to increase P uptake by more thorough exploration of soil volume thereby making positionally unavailable nutrients “available”. This is achieved by decreasing the distance for diffusion of phosphate ions and by increasing the surface area for absorption (Bowen, 1973).

Harley (1989) suggested that production of phosphatases by ectomycorrhizal fungi is important in the solubilization of organic phytates, which constitute a large fraction of total phosphate in humic soils. These enzymes are many times more active than those on non-
mycorrhizal roots (Barnett and Lewis, 1973; Mitchell and Read, 1981; Williamson and Alexander, 1975). The evidence for the induction of phosphatase activity in response to the lack of inorganic phosphate by ectomycorrhizal fungi have been given by many workers (Alexander and Hardy, 1981; Bousquet et al., 1986; Mousin et al., 1988). Similarly ectomycorrhizae have been shown to produce large amounts of calcium oxalate (Cromack et al., 1973; Lapeyrie et al., 1987; Lapeyrie, 1988; Malazczuk and Cromack, 1982) which may be involved in the chelation of Fe and Al and thereby release P for plant uptake (Graustein et al., 1977; Treeby et al., 1989).

Ectomycorrhizae are capable of increasing P uptake, especially in low fertility soils. In experiments using $^{32}$P it was found that the mantle of ectotrophic mycorrhizae accumulation remains consistent unless there is a dramatic change in the plant's P status. A phosphorus deficiency in the plant may stimulate the release by the mantle to the plant some of its accumulated P. However, whether or not an ectotrophic mycorrhiza can store other nutrients is questionable. Morrison (1962) found that pine mycorrhizae do not store sulphate while Harley and Wilson (1959) suggested that beech mycorrhiza may not store K. In many cases N uptake is also unaffected by mycorrhizae (Schenck and Hinson, 1973).

Mejstrik (1975) investigated the effect of mycorrhizal infection of Pinus abies by two Boletus species on the accumulation of Phosphorus. Following mycorrhizal development, the increase of the dry weight of roots and shoots over controls was highly significant in all combinations. The phosphorus content in mycorrhizal seedlings was higher than in non-mycorrhizal control. Ekwebelam (1979) studied the effect of mycorrhizal fungi on the growth and nutrient uptake of caribbean pine seedlings. Mitchell et al., (1984) inoculated three Quercus species with eleven isolates of ectomycorrhizal fungi and observed that mycorrhizae increased foliar nutrient content in 80
percent of the measurements. Nutrient content of K, Ca, Mg, Fe, B, Mn, Zn, Cu and Mo was also influenced by the fungal symbiont.


Rousseau et al., (1992) studied the relationship between biomass of the mycorrhizal fungus Pisolithus tinctorius and phosphorus uptake in Loblolly pine seedlings. Wallander and Nylund (1992) investigated the effects of excess nitrogen and phosphorus starvation on the extrametrical mycelium of ectomycorrhizas of Pinus sylvestris. They suggested that nitrogen deposition from the atmosphere may damage the function of mycorrhiza even before root tip studies reveal any decline in the symbiotic state.

Cumming (1993) reported that inoculation of Pinus rigida seedlings with the ectomycorrhizal symbiont Pisolithus tinctorius increased the capacity of seedlings to grow under P-limiting condition. Bucking and Heyser (1994) studied the effect of an ectomycorrhizal infection on Zn uptake and distribution depends on (1) the fungal species (2) the external concentration and (3) the Zn content of the fungal culture medium. Under conditions of low external Zn supply, especially mycorrhizal infection with Suillus bovinus led to an increased Zn uptake in roots and needles of Pinus sylvestris.

Jentschke et al., (2001) reported that the ectomycorrhizal mycelium has an active role in P acquisition from sources not available to roots. Nutrient fluxes within fungal hyphae are
interdependent and strong coupling of N, K and Mg fluxes with long-distance P translocation in the mycorrhizal mycelium occurs. Adriaensen et al., (2005) reported that the Cu-adapted Suillus luteus isolates provided excellent insurance against Cu toxicity in pine seedlings exposed to elevated Cu levels. Such a metal-adapted Suillus-Pinus combination might be suitable for large-scale land reclamation at phytotoxic metalliferous and industrial sites.

Khosla and Reddy (2008), reported that inoculation of Pisolithus albus led to the alteration in the contents of nutrient elements of plants in bauxite mined soil. Uptake of calcium and potassium was significantly enhanced in the shoots of ectomycorrhizal plants compared to nonmycorrhizal plants while their was no significant change observed in the magnesium and phosphorus contents of the plant shoots, the roots of ectomycorrhizal plants showed increased uptake of calcium and potassium than nonmycorrhizal plants but the results were not significant.

2.2. Literature on ectomycorrhiza from India:

The work on ectomycorrhizae in India has been reviewed by Lakhanpal (1987, 1988, 1989a, b, 1991) and the author has gratefully and freely drawn upon these review articles to make the present review comprehensive.

Ectomycorrhizal research in India did not get under way until the early fifties. Chaudhuri (1945) was the first to report on the mycorrhizal association in Abies spectabilis, Cedrus deodara, Picea morinda, Pinus roxburghii and Taxus baccata. However, the credit for firmly establishing mycorrhizal research goes to Dr. Bakshi, at FRI, Dehardun, who studied ectomycorrhizal association in Abies pindrow, Cedrus deodara, Picea morinda and Pinus roxburghii (Bakshi, 1957).

2.2.1. Characterization and identification:

Characterization and identification of mycorrhiza has been done in a number of plants. Bakshi (1957) studied the morphology of

Kumar and Lakhanpal (1983) reported monopodial type of mycorrhizal roots in spruce. Lakhanpal and Kumar (1984) reported brown type (Type-I) and yellowish white type (Type-II) mycorrhizae in spruce; and creamish white, yellowish white and black types of mycorrhizae in *Pinus gerardiana*. Sharma and Lakhanpal (1988) characterized and identified the mycorrhizal types in *Abies pindrow*. Verma and Shukla (1989) determined the ectomycorrhizal status of *Pinus kesiya* in the soils of Cherrapunjee. Thakur (1990) conducted studies on mycorrhizae of some conifers of Himachal Pradesh. Mehrotra and Thapar (1990) described the morphological and anatomical details of mycorrhiza in *Pinus kesiya*.

Singh (1992) studied the morphological and anatomical characteristics of ectomycorrhiza of *Cedrus deodara*. Ram (1993) studied the mycorrhiza of *Cedrus deodara* and *Pinus gerardiana* seedlings. Sagar (1993) studied the mycorrhiza of *Pinus wallichiana* seedlings.

Rawat et al., (2003) studied the vertical distribution of ectomycorrhizae in natural forests of deodar (*Cedrus deodara*) and chir pine (*Pinus roxburghii*) of central Himalayas in relation to soil properties at various soil depth (0-30cm), soil moisture, pH, organic C, organic matter, total N and available P decreased along the soil depths in both forest. Live, dead and total mycorrhizal counts were more in *C. deodara* than *P. roxburghii*. Highest number of mycorrhiza was recorded between 6-10cm depth.
2.2.2. Systematics and cultural characteristics of mycobionts:

Bakshi was tenacious in his pursuit of ectomycorrhizal research. He recorded *Scleroderma verrucosum* as the symbiont in *Eucalyptus* (Bakshi, 1966). Bakshi (1974) reported the association of *Amanita verma*, *Astraeus hygrometrious*, *Cantharellus cibarius*, *Cenococcum graniforme*, *Lactarius scrobiculatus* and *Scleroderma* spp. with different species of *Pinus* and other trees. Natarajan (1977) reported the presence of *Laccaria ohiensis* in *Eucalyptus* plantations. Sharma and Lakhanpal (1981) reported 22 species of Boletaceae found to be mycorrhizal with different trees. Natarajan and Raman (1983) described the association of *Pinus patula*, with *Scleroderma citrinum*, *Russula parazurea*, *Suillus brevipes*, *S. pallidiceps*, *S. punctatipes* and *Suillus subluteus*.

Bhatt (1986) reported *Amanita emilii*, *A. pantheriana*, *A. flavoconia*, *A. rubescens*, *A. simlensis* sp. nov. and *Russula densifolia* as mycorrhizal with *Cedrus deodar*. *Lactarius delicious* is mycorrhizal with *Picea smithiana*; *Amanita emilii*, *A. gemmata* and *Lactarius sanguifluus* are mycorrhizal with *Pinus roxburghii* and *Amanita rubescens*, *A. umbonata*, *Lactarius hygrophoroides*, *L. indicus* sp. nov., *L. piperatus*, *L. zonarius*, *Russula aurantiaca*, *R. brevipes*, *R. crustosa*, *R. lilacea*, *R. mirinoides* sp. nov., *R. subflavescens* sp. nov. and *R. subgalochroa* sp. nov. were reported to be mycorrhizal with *Quercus incana*.

Lakhanpal (1987) recorded 72 species of fungi belonging to families *Amanitaceae*, *Agarricaceae*, *Hygrophoraceae*, *Tricholomataceae*, *Russulaceae*, *Strophariaceae*, and *Paxillaceae* respectively, to be mycorrhizal with different trees in North Western Himalayas. Raman and Mahadevan (1987) and Natarajan and Purushothama (1987) reported *Lacoperdon perlateus* as a fungal symbiont of *Pinus patula*.

Kumar (1987) reported the *Amanita ceciliae*, *A. emilii*, *A. flavoconia*, *A. gemmata*, *A. pantheriana*, *A. rubescens*, *Cystoderma*
amianthinum, Lepiota clypeoparia, L. cristata, Macrolepiota sp. nov.,
Tricholoma virgatum to be mycorrhizal with Cedrus deodar. Agaricus
angustus, Amanita caesarea, A. Rubescens, Clitocybe gibba, Collybia
fusipes, Hygrocybe sp. nov., Leucoagaricus rubratinctus with Quercus
incana; Hygrophorus chrysodon, H. pudorinus, H. pudorinus var.
fragrans, Leucopaxillus amarus, F. roseibrunneus, L. giganteus with
Picea smithiana; Amanita berkeleyi, A. emilli, A. gemmata, A. vaginata
with Pinus roxburghii; Clitocybe clavipes, Laccaria laccata, L.
amethystea with Pinus wallichiana; Lepista nuda, Amanita volvata with
Quercus semicarpifolia; Hygrophorus eburneus with Rhododendron
arboretum and Amanita fulva with Betula sp.

Singh and Thapar (1988) identified 29 fungal symbionts which
were in intimate association with different plant species and presented
a diagonalistic key for their identification. Raman and Mahadevan
(1988) reported results on the selection of fungi for ectomycorrhizal
inoculation in Pinus patula, Amanita muscaria, Laccaria laccata,
Lycoperdon perlatum and Scleroderma citrinum were described to be
mycorrhizal with Pinus patula. Sharma and Singh (1990) observed
that Lactarius sanguifluus, Suillus sibiricus, Boletus edulis and
Thelephora terrestris formed symbiosis with Pinus roxburghii.

Singh (1992) identified and described taxonomical details of
Trappeinda himalayansis gen. et sp. nov. associated with Cedrus
deodara. Sagar (1993) identified and described taxonomical details of
Suillus sibiricus associated with Pinus wallichiana.

Trappe et al., (1994) recorded for the first time some rare
hypogeous fungi from India, from N.W. Himalayan ranges. These were
Tuber mesentricum, Rhizopogon rubescens var. ochraceus,
Melanogasteur broomeanus, Gauteria trabuti and Hysterangium
membranaceum. This is the first authentic report of tuber from the
country. Kumar et al., (1999) reported association of Scleroderma sp.
with Acacia sp. from Andhra Pradesh.

Jha et al., (1990) recorded maximum colony growth in Laccaria laccata at pH 5 on Melin-Norkran’s medium. Collybia radiata and Russula luteolus grew at pH 6 and Pisolithus tinctorius at pH 7. Modified Melin Norkran, s medium (MMN) Norkran’s medium (NM) and Hagem’s medium (HM) were tested to find a suitable medium for mass multiplication of P. tinctorius. MMN was the best medium that promoted maximum colony diameter and dry weight of fungi (Rangarajan et al., 1990). Laccaria laccata and Amanita muscaria were grown at 20, 24, 28, 32 and 36°C, both in the light and in the dark. Laccaria laccata and A. muscaria grew well at 24°C and 28°C respectively and in total darkness (Raman and Thiagarajan, 1988). Wild strains of L. laccata grew well at 24 and 30°C and mutant strains grew well at 24, 30 and 36°C (Raman, 1990).

Singh (1992) isolated Trappeinda himalayansis gen. et sp. nov. into pure culture and studied its physiological characteristics for different carbon sources, nitrogen sources, vitamins trace elements and growth regulators. Sagar (1993) isolated Suillus sibricus into pure culture and reported Hagem’s medium as the best medium for its growth.

Sundari and Adholeya (2003) studied fungal isolates to determine the pH optima for growth, substrate acidification by the culture and effect of substrate acidification on culture growth. Of the isolates tested, the members of Agaricales (except Laccaria laccata) and Aphyllophorales favored neutral to near neutral pH, while members of the order Sclerodermatales strictly favored acidic pH. Laccaria laccata was the fastest growing culture of the tested isolates,
completing growth in week. This cultured favored an acidic pH (6.0) for optimal mycelial growth.

2.2.3. **In vitro synthesis and inoculation with ectomycorrhizal fungi:**

Not much has been done in India in this regard but whatever has been done holds a good promise for future. Bakshi (1974) synthesized mycorrhiza of *Pinus patula* with *Sclerodema qeaster*. Kanan and Natarajan (1987, 1988) synthesized ectomycorrhiza of *Scleroderma citrinum* and *Amanita muscaria* with *Pinus patula*. Raman (1988) reported the mass production of ectomycorrhizal spawn of *Laccaria laccata* and *Amanita muscaria* in sorghum grains. Kumar and Lakhanpal (1991) reported results of artificial inoculation of *Pinus gerardiana* seedlings with mycorrhizal associates isolated from mycorrhizoplane of natural roots. Singh (1992) reported *in vitro* synthesis of ectomycorrhizae between *Cedrus deodara* and *Trappeinda himalayansis*. Sagar (1993) achieved *in vitro* synthesis of ectomycorrhizae between *P. wallichiana* and *Suillus sibiricus*.

Reddy and Satyanarayana (1998) studied five ectomycorrhizal fungi, *Cenococcum geophilum, Laccaria laccata, Paxillus involutus* and two isolates of *Pisolithus tinctorius* used to inoculate micropropagated plantlets of *Populus deltoides* to produce the mycorrhiza *in vitro*. *Paxillus involutus* formed mycorrhizas with plantlets of *Populus deltoides* while other failed.

Reddy et al., (2002) reported the inhibitory effect of Aluminum on the growth and mineral nutrition of *Cantharellus cibarius* and *Pisolithus sp*. Sagar and Lakhanpal (2005) achieved *in vitro* synthesis of ectomycorrhizae between *P. wallichiana* and *Suillus sibiricus*.

2.2.4. **Studies on Physical and chemical status:**

The mycorrhizal plants were reported to attain better shoot/root ratio (Fresh weight and dry weight) and to exhibit higher shoot root ratio compared to non-mycorrhizal plants. Marked differences in
needle nutrient content have been observed with higher concentration of P, Ca and Mg (Kumar and Lakhanpal, 1983). In a similar study on Cedrus deodara, Singh (1992) reported considerable improvement in growth and development, and nutrient uptake of the seedlings inoculated with Trappeinda himalayensis. Sagar (1993) reported considerable improvement in growth and development, and nutrient uptake of the Pinus wallichiana seedlings inoculated with Suillus sibiricus.

Singh and Lakhanpal (2000) introduced three types of inocula in Cederus deodar bare root nursery after fumigation with formaline to evaluate them for their mycorrhization capacity. The inocula consisted of pure culture of Boletus edulis and Russula brevipes, crushed sporophores of these two species and naturally colonized soil of cedrus deodara forest. Out of the two pure culture inocula, B. edulis formed higher percentage of ectomycorrhizal roots (83.16%) compared to R. brevipes (70.00%). Natural soil inoculum produced (57%) ectomycorrhizae and percentage was higher than the crushed sporophore of B. edulis (45.50%) and R. brevipes (41.3%).

Pande et al., (2007) showed that the outcome of competition between the seedlings of two major Indian Himalayan tree species, viz. ban oak and chir pine is changed with the change in ectomycorrhizal fungal species. Both in oak and pine, the seedlings inoculated with ectomycorrhizal fungi showed significantly more growth in all parameters (shoot length, root length, collar diameter) than the uninoculated ones. The ectomycorrhizal fungi, Russula and Amanita, significantly increased the number of fine roots. While oak does better than pine when grown in a mixed culture in the presence of Russula vesca, the outcome was reversed in the presence of Amanita hemibapha.

Khosla and Reddy (2008) reported that ectomycorrhizal plants showed better growth and survival when compared to nonmycorrhizal plants in bauxite mined soil. Pisolithus albus colonized 57.8% of the
laterals roots. The mean plant height of the ectomycorrhizal plants was significantly higher than the nonmycorrhizal plants. The dry weights of shoot and root were significantly more in ectomycorrhizal plants than nonmycorrhizal plants. The shoot/ root ratio was significantly lower for the ectomycorrhizal plants as compared to nonmycorrhizal plants.