STUDIES ON THE CLIMATIC FACTORS ON THE DEVELOPMENT AND EFFICIENCY OF ECTOMYCORRHIZAE OF PINE

(Pinus kesiya Royle Ex. Gordon*)*

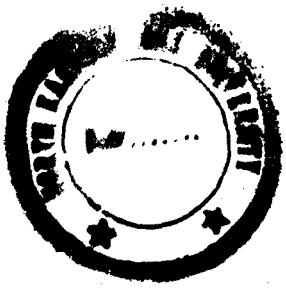
ABSTRACT

By

BIRENDRA NATH JHA

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF

DOCTOR OF PHILOSOPHY IN BOTANY



To

NORTH-EASTERN HILL UNIVERSITY

SHILLONG (INDIA)

FEBRUARY, 1990

Box
(Thesis)

A study on the seasonal and spatial distribution of ectomycorrhizae and the mycobionts situated at different altitudes of Khasi Hills was done. Fifteen ectomycorrhizal fungi were observed during the whole period of investigation in the vicinity of 2, 7 and 12 years old pine plantations. Maximum number of ectomycorrhizae and their sporocarps were observed at the lower altitude and it decreased with the increase in altitude.

Age of pines influenced the population of sporocarps and ectomycorrhizae which was found to be directly proportional to the age of the stands. Middle rainy season was most conducive for the development of sporocarps. No sporocarp was observed during winter months.

Diversity index of the fungal symbionts was maximum at the lower altitude and minimum at the upper altitude. Boletus sp., Lactarius sp., Russala sp. and Amanita sp. were early successional species at the lower altitude except Lactarius sp. Boletus sp. was found occurring throughout the whole growing season at all the three altitudes.

Positive correlations were obtained between the ectomycorrhizal population and the climatic as well as edpahic conditions in majority of the pine stands at different altitudes. At the higher altitude a negative correlation was obtained between the relative humidity and ectomycorrhizal

population in 12 years old pine stand in the second year of investigation (1988). Similar result was observed between relative humidity and mycorrhizal population in 1988 at the middle altitude in 7 years old stand. Ectomycorrhizae were found negatively correlated with the rainfall in 2 years old stand at the lower altitude in 1988.

Isolation of mycorrhizal fungi, their maintenance, mass inoculum preparation and pure culture synthesis of mycorrhizae with pine (Pinus kesiya Royle ex. Gordon) were done. Pisolithus tinctorius, Scleroderma aurantium, Cenococcum sp., Boletus edulis and Suillus sp. were isolated in pure form and their mycorrhizae were synthesized using these fungi on synthetic media which confirmed their symbiotic relationship with pine roots. Cenococcum sp. was isolated from the mycorrhizal roots while other fungi were isolated from the fungal sporocarps. Digitate and black mycorrhizae were formed by Cenococcum sp. while coralloid type of mycorrhizae were formed by Boletus edulis. Other fungi formed dichotomously branched mycorrhizae.

Pure mycelial inoculum was prepared for their use in experimental purpose.

Effect of climatic conditions i.e., temperature, relative humidity and light intensity and pH were investigated on the growth of Laccaria laccata, Collybia radicata, Rhizopogon luteolus and Pisolithus tinctorius.

Most of the fungi grew well at 25°C but R. luteolus and P. tinctorius were found at 20°C and 30°C respectively.

High humidity level favoured the growth of most of the fungi on solid medium, however, the growth of P. tinctorius was favoured by the low level of humidity.

Dark condition favoured the growth of all the ectomycorrhizal fungi and it decreased with the increase in intensity of light. L. laccata attained maximum diameter followed by C. radicata and R. luteolus while P. tinctorius produced minimum colony spread at lower light intensity.

Most of the fungi grew well between 5 to 6 pH range. R. luteolus and P. tinctorius showed little affinity towards acidic and alkaline conditions respectively.

Acid phosphatase activity of ectomycorrhizal fungi was maximum at 30°C, low humidity, light condition and 3- pH_e.

Acid phosphatase activity was always more than alkaline phosphatase activity.

Effect of temperature, light and humidity on the colonization of mycorrhizae and uptake of phosphorus and nitrogen by pine seedlings were also studied. High and moderate light intensities favoured the colonization of ectomycorrhizae and efficiency of uptake of nitrogen and phosphorus. However, at 10°C temperature uptake of nitrogen and phosphorus and colonization potential of mycorrhizal fungi were lower.

Relative humidity did not show significant variation in colonization of mycorrhizae as well as uptake of nitrogen and phosphorus. Pine seedlings infected with P. tinctorius attained maximum growth compared to others.

Survival of the pine seedlings was higher under moderate light intensity than high light intensity. Lowest survival of seedlings was observed under low light intensity. P. tinctorius enhanced the survivorship of seedlings more efficiently than other fungi. Uninoculated seedlings showed minimum survival percentage at all the light regimes.

Percentage survival of the seedlings was lowest at 30°C and highest

at 25°C.

Low level of humidity decreased the survivorship of the seedlings compared to high level and it was noticed miminum with L. laccata and maximum with P. tinctorius at low level of humidity.

Different doses of phosphorus affected the growth and mycorrhizal colonization of the pine seedlings. Double dose of the phosphorus (2P) favoured the growth of the seedlings in general at high and moderate light intensities while there was no such effect of 2P level of phosphorus under low light intensity, however, the growth of seedlings infected with R. luteolus was more with 1P level than other levels under high light intensity.

1/2P level of phosphorus was found favourable for mycorrhizal colonization and the growth of pine seedlings at 25°C and 10°C. Insignificant difference in colonization of mycorrhiza and seedling growth was observed between 1P and 2P levels of soil phosphorus. 1/2P level of phosphorus also favoured the intensity of mycorrhizal colonization and growth of pine seedlings at both the himidity levels (low and high).

Survival of the seedlings was also affected by the different levels of phosphorus. Surival of seedlings was reported highest at 1/2P level of soil phosphorus compared to 2P and 1P levels at 25°C and 10°C temperatures. Similar result was observed at low and high levels of relative humidity.

Concentrations of phosphorus and nitrogen were found different in the shoots and roots of pine. Root phosphorus concentration was noticed quite high in 2P supplied set at high light intensity. Seedlings with 2P level were found more efficient in phosphorus absorption under moderate light intensity compared to high light intensity. Uptake of nitrogen was also

more at high and moderate light intensities. No significant difference in the concentrations of phosphorus and nitrogen were observed between the infected and uninfected seedlings at different levels of temperature and humidity.

Alkaline phosphatase activity was found reduced in all the cases than acid phosphatase activity. High and moderate light intensities increased the root phosphatase activity. Similarly enhanced activity of root surface phosphatase was found at 25°C. Insignificant variation in the surface phosphatase activity of the mycorrhizal roots was observed at different levels of humidity.

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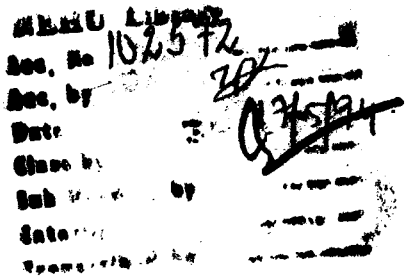

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February, 1990

CERTIFICATE

We certify that the thesis entitled **Studies on the climatic factors on the development and efficiency of ectomycorrhizae of pine (Pinus kesiya Royle ex. Gordon)** submitted by Mr. Birendra Nath Jha, for the degree of **Doctor of Philosophy** of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under our supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. degree. This work has not been submitted for any Degree of any other University.

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Birendra Nath Jha.

Dated, 9th March, 1990

(BIRENDRA NATH JHA)

CONTENTS

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Khasi pine (Pinus kesiya Royle ex. Gordon), an indigenous timber yielding species is dominant at the higher altitudes of foot hills of North-Eastern Himalayas. It grows luxuriantly as an early successional tree species of natural forest ecosystem. Under natural conditions, the roots of pine form a symbiotic association with fungi termed as 'Mycorrhiza' (Frank, 1885). The mycorrhizal association helps in the establishment and survival of the seedlings of pine (Marx, 1975; Marx et al., 1976; Sharma, 1981; Bledsoc et al., 1982; Valdes, 1985; LaBuglio and Wilcox, 1988). The symbiotic association enhances the physiological activity of the root tissue system and also the absorption of mineral nutrients from the soil (Hatch, 1937; Harley, 1960; Bowen and Rovira, 1969; Rovira and Bowen, 1971).

Various types of mycorrhizal associations, have been reviewed (Lewis, 1973, 1975, 1976; Read, 1982) and broadly grouped into three major groups (ectomycorrhiza, endomycorrhiza and ectendomycorrhiza). Pines have ecto- or ect-endomycorrhizal association formed mainly by basidiomycetes, hypogeous ascomycetes, phycomycetes and deuteromycetes (Gerdemann, 1974; Trappe, 1962, 1977).

The success of an introduced plant species depends upon a number of ecological factors (Grime, 1979). The mycorrhizal association, especially the ectomycorrhiza type, is adapted to nutrient stresses and is an ecological aid to plant species growing in a temperate condition (Mexal, 1980

However, considering the plant communities as a whole, the ectomycorrhizal association is found only in 3% (Meyer, 1973) or 5% plants (Mexal, 1980), while majority of the plants possess vesicular-arbuscular type of mycorrhiza (Gerdemann, 1975; Mexal, 1980).

Ectomycorrhizal fungi enhance absorption of inorganic nutrients, particularly phosphorus (Theodorou, 1968, 1971; Ho and Zak, 1979), produce growth hormones (Moser, 1959; Ulrich, 1960; Miller, 1967, 1971; Gogala, 1967, 1971; Slankis, 1973), growth regulators (Shamakhanova, 1962; Turner, 1962), check the root pathogens (Perrin, 1985; Duchesne et al., 1987, 1988, 1989), decrease soil toxicity and increase resistance to extreme soil temperature (Lapeurie et al., 1984) and drought conditions (Parke et al., 1983; Heinrich et al., 1988).

Climatic factors may affect the growth and development of ectomycorhizal fungi. Some information has been acquired on inter and intra specific growth variation of ectomycorrhizal fungi in response to different temperatures (Moser, 1958; France and Reid, 1979; Cline et al., 1987). The studies carried out in laboratory condition on ectomycorrhizal fungi may, however, show difference in response to natural environmental conditions due to complexity and interaction between soil, climatic and biological components (Mikola, 1948).

pH may also affect the growth of ectomycorrhizal fungi (Norkrans, 1949). These fungi exhibit a broad or narrow range of tolerance (Theodorou and Bowen, 1969; Laiho, 1970; Hung and Trappe, 1983; Stroo and Alexander, 1985; McAfee and Fortin, 1987). The probable reasons affecting their growth in terms of enzyme activity is, however, seldom attempted (Ho and Zak, 1979; Dighton, 1983). Studies on acid phosphatase activity together with the physical factors may provide some clues to explain the growth pattern of ectomycorrhizal fungi.

Similarly, some experiments on the water supply show that ectomycorrhizal fungi could survive in water deficient soils (Uhlig, 1972; Malabari, 1979; Parke

et al., 1983; Heinrich et al., 1988) and can translocate the stored water to the host (Duddrige, 1980).

Light intensity also has effects on the growth of ectomycorrhizal fungi (Ashton, 1976; Son and Smith, 1988).

The role of ectomycorrhizal fungi in nutrient uptake has widely been attended, but the role of climatic and edaphic conditions on the development of mycorrhiza is very less studied. Until the end of the 19th century, it was believed that plants with ectomycorrhizae absorb more nitrogen than non mycorrhizal plants (Frank, 1894). Later on, the work of Hatch (1937), Mitchell et al. (1937) and Finn (1942) strengthened the view that ectomycorrhizal seedlings of pine contained greater quantities of nitrogen, potassium and phosphorus than the non mycorrhizal seedlings (Ashford et al., 1975; Edmonds et al., 1976; Chilvers and Harley, 1980; Bowen and Smith, 1981; Harley and McCready, 1981; Strullu et al., 1981, 1982; Alexander, 1982; Heinrich and Patrick, 1985; Heinrich et al., 1988). Work on phosphatase activity of ectomycorrhizal roots has also been extensively undertaken (Williamson and Alexander, 1974; Antibus et al., 1981). Little work on the physical factors affecting the pine seedlings and development of ectomycorrhiza are available. Light intensity (Hatch, 1937) and moisture content of the soil (Reid, 1979) can encourage ectomycorrhiza formation, while studies on the effect of climatic factors on the development of mycorrhiza are needed.

The problem of environmental degradation is very complex. Extensive industrialization, urbanization and increased human population have adversely affected the forest ecosystem. Large scale deforestation has been changing the global climatic conditions. However, high precipitation and hilly topography with less vegetation have led to the formation of nutrient poor soil in the area.

The changing climatic and edaphic conditions due to continuous deforestation provoked to take up the present study on ectomycorrhizae of pine on the following aspects.

- Spatial distribution of ectomycorrhizal fungi in pine stand at different altitudes of Khasi hills, Meghalaya.

- Isolation technique, maintenance and mass culture of ectomycorrhizal fungi.

- Effect of climatic factors on the growth behaviour of ectomycorrhizal fungi in vitro conditions.

- Effect of climatic conditions on the development of ectomycorrhizae.

- Nutrient status (N and P) of pine seedlings and phosphatase activity of ectomycorrhizal roots of pine under different climatic conditions.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Khasi pine (Pinus kesiya Royle ex. Gordon) is an indigenous timber yielding species which grows luxuriantly under natural condition in North-Eastern region of India. It is also an early colonizer among the tree species of evergreen forest of Khasi hills of Meghalaya. Under natural conditions, the roots of pines are associated with fungi. Such an association was first observed by Unger in 1840. Later on Reissek (1847) noticed the hyphae in the cells of many angiosperms and Komienski (1881) showed a complete fungal layer around the roots. But it was the German Botanist A.B. Frank (1885), who coined the term **mycorrhiza** ('mycos' = fungus; 'rhiza' = root) for this type of association. He assumed this association as symbiotic one. Recently, Marks and Foster (1973) concluded after cytological studies of different fungi that mycorrhizal association is truely a symbiotic one like lichens.

Studies on mycorrhizae have got a momentum due to their importance in mineral nutrition of plants. Komienski (1881) was the first to point out that all the material absorbed from the soil must pass through the fungal layer formed around the roots of many angiospermic plants. Later, in 1894, Frank proposed the **Nitrogen Theory** which expressed that mycorrhizal plants were capable of absorbing organic nitrogen from the nitrate deficient soils.

Mycorrhizae have been classified into three major groups i.e., ectomycorrhiza, endomycorrhia and ect-endomycorrhiza (Lewis, 1973, 1975, 1976, Read, 1982).

Singer and Morello (1960) have reported that ectomycorrhizal plants occur at higher altitudes of the region of 25'N and 35.55'E.

Peglar and Fiard (1979) have reported that ectomycorrhiza are most prevalent in areas having variable seasons.

Harley and Smith (1983) have the opinion that ectomycorrhizal association may exist in areas where vegetative activity is restricted during some period of the year for edaphic, climatic or other environmental reasons.

Distribution of ectomycorrhizal fungal species in nature

(i) Survey of the fungi in nature

Trappe (1962, 1977) has estimated over 2000 fungal species as potential ectomycorrhizal symbionts in natural condition. Most of these species have been identified by their fruit bodies. However, the production of sporocarps depends upon the environmental conditions of the area (Sharma, 1981).

Lamb (1979) has reported 40 ectomycorrhizal species in Pinus elliottii stand.

Sharma (1981) has reported 33 fungal species in Khasi hills of Meghalaya with Pinus kesiya.

Hedger (1982) has the similar observation with the production of fungal sporocarps.

Thomas et al. (1983) reported 24 different fungi with Pinus sitchnsis.

(ii) Population dynamics of fungal sporocarps related to age of the host

Trappe and Strand (1969) have reported that fungi like Thelephora

terrestris, Laccaria, Hebeloma and Inocybe spp. were associated with the young trees.

Usher and Parr (1977) also reported that diversity of fungal species increased with the increase in the tree age and declined in thick canopy.

Chu-chou (1979) also noted the similar observation in coniferous forest.

Last et al. (1981) observed that increase in number of fruit bodies of Amanita muscaria was directly correlated with the increased age of Pinus patula tree.

Sharma (1981) and Chu-chou and Grace (1981, 1983) have also showed the similar observation. They reported that sporocarp production and species occurrence were significantly influenced by the age of the host.

Mason et al. (1982) observed a change in fungal diversity with stand development. They found that Lactarius rufus was dominant in young tree stand while a diverse group of fungi occurred in a close climax community. They further reported that fruit bodies of Paxillus involutus have not been observed in young trees growing in a brown earth indicating possible selection by the environmental conditions.

In 1983, they studied the spatial and temporal shift of ectomycorrhizal fungal fruit bodies in a birch stand.

Last et al. (1983) observed occurrence of Hebeloma crustuliniforme and Laccaria tortilis fruit bodies within 2 years of birch planting, Inocybe lanuginella and Lactarius putescens after 4 years, species of Cortinarius and Leceinum after 6 years and Russula sp. after 10 years of planting.

Deacon et al. (1983) examined the succession of mycorrhizal fungi and distribution of mycorrhizae in 8 years old Oak tree community. Hebeloma type mycorrhiza was most frequent in the outer periphery with newest part

of the root system, while Leceinum type mycorrhiza was mostly found in the inner core of the stand.

In another study they indicated that the ability of fungi to be as either early or late in the sequence of mycorrhizal establishment was determined by the field conditions.

The successional pattern of the fungi to establish mycorrhizae was further confirmed by Fox (1983). He synthesized the mycorrhiza with the basidiospores of late stage mycobionts under amended unsterile soils.

Fleming (1983) established that L. pubescens formed mycorrhizal association with birch seedlings when transplanted in contact with the root system of a parent tree which otherwise failed to do so.

Garrett(1951) and Fleming (1983) have also reported that removal of early stage fungi from a system reduces the infection potential of host.

Deacon et al. (1983) explained that early stage fungi were less glucose-demanding than late stage fungi which was later on supported by them.

Isolation, maintenance and mass inoculum production

Several criteria have been used to identify the fungal partners of ectomycorrhizae with varying degree of success.

Peyronel (1922) compared the mycelium at the base of the sporocarp with fungal tissues attached to underlying mycorrhizae.

Woodroof (1933) traced the connecting rhizomorphs from mycorrhiza to sporocarp.

Zak and Marx (1968) and Zak (1969) have traced the connections between pine mycorrhiza and sporocarp via mycelium. However, this was not sufficient enough to prove the symbiotic association between the fungi

and the host.

Warcup (1971) isolated 25 fungi from their sporocarps growing in eucalypt forest and identified them as mycorrhizal one.

Lamb (1979) isolated 20 different species from the sporocarps of P. radiata stand.

Sharma (1981) following the above method isolated more than 10 fungal species from the P. kesiya stand. Ng et al. (1981) have also isolated R. luteolus and B. elegans from the sporocarps collected from P. radiata woodland.

Malajczuk et al. (1982) isolated some fungi from sporocarps. They isolated Cenococcum geophilum from the sterilized sclerotium.

Zak (1969, 1971) found potato dextrose agar medium formulated by Lacy and Bridgmon (1962) and modified Melin-Norkrans medium as best media for fungal growth.

Chu-chou (1979), Sharma (1981) and Chu-chou and Grace (1988) used different media to isolate the fungi from the mycorrhizal roots and suggested that Hagem (Modess, 1941) and MMN (Marx, 1969) agar media were best for the fungal growth.

Hacskaylo (1953), and Marx and Zak (1965) synthesized the mycorrhiza between fungal partners and the host under controlled condition.

Pachlewska (1968) augumented that a starvation medium consisting of agar, thiamine and water was more suitable for the synthesis of mycorrhiza with P. sylvestris seedlings.

Sharma (1981) also used the agar thiamine medium and synthesized

mycorrhizae.

Fast multiplication and mass production of ectomycorrhizal fungi on large scale is necessary to inoculate the seedlings in nurseries, afforestation and plantation programmes.

Moser (1958) attempted to produce ectomycorrhizal fungal inoculum using the pure mycelial culture.

Bowen (1965), Mikola (1973), Trappe (1977) and Marx (1980) have used the pure mycelial cultures for inoculation of the seedlings of trees.

Park (1969) used wheat grains to prepare the inoculum of Suillus granulatus and Cenococcum graniforme for artificial inoculation.

Marx et al. (1984) tried both liquid and solid cultures to produce mass inoculum.

Sahardi (1988) suggested a spore inoculum technique for pine stand under natural condition.

Sharma and Mishra (1988) were of the opinion that fast growing fungi can produce more inoculum within short duration. They improved the growth of fungi by adding Sphagnum to the MMN vermiculite medium and found it better over other sources of inocula.

Raman (1988) found Sorghum grains superior than wheat grains for the growth of fungus.

Effect of climatic conditions on the growth of fungi

Several criteria have been used to select ectomycorrhizal fungi for inoculating them in nursery soils (Trappe, 1977). Some of these are the temperature, pH, light and humidity.

(i) Temperature

Mikola (1948.) pointed out that the ambient temperature in the natural environment of mycorrhizal fungi was often less than their optima observed in culture.

Hacskaylo et al. (1965) have shown that there is variation in temperature reaction between the strains of one species and the same strain on different media.

Harley (1969) studied the effect of temperature on the growth of mycorrhizal fungi in culture and suggested that a temperature range between 18°C to 27°C was optimum for the growth of mycobiont, whereas Moser (1956) had shown a better growth of Suillus plorans at 15°C to 18°C.

Laiho (1970) investigated that strains of Paxillus involutus could grow between a range of 5 to 32°C with optima between 15 to 25°C.

Marx et al. (1970) established a linear relationship between dry weight of the mycobionts and the temperature.

Theodorou and Bowen (1971) have also obtained the similar linear relationship between the growth of fungi and temperature.

France and Reid (1979) advocated that some isolates of mycorrhizal fungi can withstand very low temperature (-10°C) for shorter period. However, chilling treatment affected their recovery period to attain the normal growth.

Cline et al. (1987) observed maximum growth of Cenococcum geophilum and Suillus granulatus at 27°C and of Pisolithus tinctorius at higher temperature (32°C).

Peng and Chien (1988) studied the effect of temperature on the growth

of ectomycorrhizal fungi *in vitro* and observed different optima levels for different fungi.

(ii) pH

Melin (1924) and Modess (1941) opined that ectomycorrhizal fungi always grow in acid side of the neutrality. They generalized that all ectomycorrhizal fungi are acidophilic in nature.

Modess (1941) observed that pH was one important factor for the growth of mycorrhizal fungi.

How (1940) observed the optimum pH range for *Boletus elegans* from 4 to 5.5.

Bokor (1959) reported good growth of many species of mycorrhizal fungi between pH range of 6.8 to 8.3.

Mikola (1962) reported the reduced production of pigments of the ectomycorrhizal fungi at extremes pH *in vitro*.

Harley (1969) has reported that acidic pH was better for the mycelial growth of mycorrhizal fungi.

Laiho (1970) found that strains of *Paxillus involutus* might have optimum growth between pH 3.1 to 6.4 and the pigment production was reduced at extreme pH levels.

Giltrap and Lewis (1981) were of the opinion that pH of the medium may affect the fungal growth. They observed more dry weight of *Cenococcum geophilum* in buffered nutrient solution above pH 5 than unbuffered solution.

Harley and Smith (1983) expressed that the change in pH may not be due to absorption of nitrogen compounds, but a result of organic acids

which may be inhibitory. However, it needs further investigation, particularly in relation to the nutrient absorption.

Hung and Trappe (1983) reported the variation in growth among the different strains of the same species.

Peng and Chien (1988) reported an excellent growth of Boletus griseus both at acidic and neutral pH conditions.

Jha et al. (1990) reported interspecific growth variation in response to the different pH.

(iii) Light and relative humidity

Harley (1969) and Harley and Smith (1983) were of the opinion that light was an important factor for the growth of ectomycorrhizal fungi in symbiotic conditions.

Bowen (1964) studied the effect of relative humidity on the growth of mycorrhizal fungi. He reported better growth of C. geophilum at much lower relative humidity while Bowen and Theodorou (1973) showed that the growth of R. luteolus ceased in relative humidity approximating those of soil at wilting point in laboratory media.

Mexal and Reid (1973) studied the effect of water stress on three ectomycorrhizal fungi.

Uhlig (1972) pointed out that mycorrhizae with spruce, survived below water deficits level and induce drought resistance in host than their uninfected counterparts.

Recently Duddridge et al. (1980) have examined the functionality of the rhizomorphs of Suillus bovinus in the transport of water to Pinus sylvestris. They showed that the rhizomorphs contained differentiated 'vessel'

hyphae between 20 and 60 μm, in diameter in which the transverse walls were broken down.

Peng and Chien (1988) observed the xerophile nature of two species of Boletus and Suillus which could grow in higher water stress conditions.

Effect of climatic conditions on the mycorrhizal development and nutrient (N and P) uptake

(i) Light

Hatch (1937), on the basis of experimental results, suggested that internal nutrients status, especially with respect to nitrogen, phosphorus and potassium, was the prime factor in determining the intensity of infection. He reported that the infected roots in high light intensities were dependent upon the internal nutrient status of the soil.

Bjorkman (1942) found that light intensity exerted considerable effects on mycorrhizal development. He found the mycorrhizal infection directly proportional to the increased light intensity. Using the natural soils he experimented with the added nutrients and concluded that a severe lack of available nitrogen or phosphorus, hampers the formation of mycorrhiza as well as growth, but moderate scarcity of one or other of these nutrients is a condition for mycorrhizal infection.

Mikola (1948) had reported the higher frequency of mycorrhiza at 10% day light with C. graniforme.

Wenger (1955) and Harley and Waid (1955) showed that the mycorrhizal development increased with increase in light intensity.

Hacskaylo and Snow (1959) studied the effect of duration and intensity of light upon mycorrhizal development in soils of different nutrient levels.

Boullard (1961) showed that an increase of light period from 6 hrs

to 16 hrs, increased the development of mycorrhizae.

Mosse (1973) reported that mycorrhizal growth response might be reduced due to accumulation of P to toxic concentrations in the mycorrhizal plants at low light intensity.

Hayman (1974) suggested that the efficiency of P uptake might be reduced at low irradiance and this could lead to P deficiency and reduced growth responses.

Bevege et al. (1975) showed accumulation of labelled carbon (photosynthetically produced) in mycorrhizae 15 times more than in uninfected roots of P. radiata .

Stribley et al. (1980), Buwald and Goh (1982), Bethlenfalvay and Pacovsky (1983) and Tester et al. (1985) observed reduced growth response at low irradiance and high soil phosphorus.

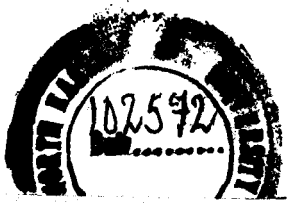
Bhat (1982), Tester et al. (1985), and Smith et al. (1986) have shown that P uptake can be reduced at low light intensities.

Bethlenfalvay and Pacovsky (1983) has shown that light and high soil P had a direct effect on source-sink relationship.

Son and Smith (1988) suggested the interaction between photo irradiance and phosphorus uptake by mycorrhizae. They found that there was positive mycorrhizal response at high irradiance grown without additional P, but at low irradiance the mycorrhizal growth was also low.

Finlay (1989) has shown extensive translocation of labelled assimilate to the fungal mycelium in P. sylvestris seedlings infected with Boletus cavipes.

Cariney et al. (1989) studied the distribution of fixed photosynthate



in root systems of Eucalyptus pilularis infected with P. tinctorius. They found increased amount of photosynthetically labelled carbon accumulated in the root system than non-mycorrhizal roots.

(ii) Temperature

Redmond (1955) observed that either decreasing or increasing the soil temperature by 2°C resulted changes in thickness of the fungal mantle.

Carrodus (1965) observed that uptake of ammonium was slightly better between 10°C to 20°C which was decreased at 20°C to 25°C.

Harley (1969) studied the effect of temperature on the rate of uptake of nutrients and found a slow uptake rate of nitrogen and phosphorus at 0°C which was increased upto 15°C to 20°C.

Marx et al. (1970), Marx and Bryan (1971), Theodorou and Bowen (1971), and Dixon et al. (1981) have stated that influence of temperature on the development of mycorrhizae varied from species to species.

Bowen (1970) indicated that change in soil temperature altered the root exudation.

Theodorou and Bowen (1971) have extensively studied the effect of temperature on colonization of P. radiata roots and stated experimentally that the length of root colonized was significantly less at 16°C soil temperature.

Theodorou and Bowen (1971) observed a rapid decline in mycorrhizal intensity alongwith decreasing the temperature below 20°C.

In another study during 1973, they have stated experimentally using Suillus sp. and R. luteolus, that the length of root colonized was significantly less at 16°C soil temperature than at 25°C and in case of Suillus granulatus

less than 20°C.

Parkeet al. (1983) have observed that optimal temperature for mycorrhizal formation in Douglas-fir was 18.5°C in undisturbed forest soil and 24°C in clear cut soil.

(iii) Moisture content

Worley and Hacskaylo (1959) showed that mycorrhizal formation in P. virginiana seedlings was affected by moisture content of the soil. The frequency was greatest in moist soil and least in dry condition. In addition, the type of mycorrhizal development was different in moist and dry soils.

Bowen and Theodorou (1973) observed that colonization of P. radiata by R. luteolus, declined markedly above field capacity and below 50% field capacity soil moisture.

Read et al. (1977) suggested that formation of ectomycorrhizae by Helianthenum chamaecistus with Cenococcum ssp. was an adaptation to dry soil condition.

Reid (1979) reviewed the effect of water stress on mycorrhizal fungi which vary according to their ability to withstand low water potential in soil.

Harley and Smith (1983) stated that soil moisture may affect the ability of mycorrhizae either at high water potential effecting the aeration or at low water potential causing water stress condition.

Phosphatase activity

(i) Of ectomycorrhizal fungi

Ho and Zak (1979) analysed the acid phosphatase activity of Laccaria laccata, Hebeloma crustuliniforme, Amanita muscaria, Thelephora terrestris,

Rhizopogon vinicolor and Piloderma bicolor and concluded that their hydrolysing capacity of PNPP was different.

Dighton (1983) reported production of acid phosphatase by a number of fungi and showed that L. rufus and P. involutus hydrolysed more IHP-P than Suillus luteus.

Ho and Tilak (1988) used the paper disk method for scanning large numbers of ectomycorrhizal fungi. They compared their findings to the findings of Ho and Zak (1979) and found almost similar.

(ii) Of mycorrhizal roots

Bartlett and Lewis (1972) discovered that the rate of hydrolysis of orthophosphate on mycorrhizal root tips was greater than the rate of its uptake resulting into a net appearance of inorganic phosphate.

Antibus et al. (1981) measured the surface phosphatase activity of mycorrhizal complexes of Salix rotudinfolia. They observed that acid phosphatase activity was highest with Entoloma sericeum infected roots followed by C. geophilum and Hebeloma pasillum.

Alexander and Hardy (1981) studied the surface phosphatase activity of Sitka Spruce mycorrhizae from a serpentine site.

Dodd et al. (1987) recorded more acid phosphatase activity on mycorrhizal roots than non-mycorrhizal one.

CLIMATE, VEGETATION AND SOIL OF THE STUDY SITE

CLIMATE, VEGETATION AND SOIL OF THE STUDY SITE

Pine forest of different ages (12, 7 and 2 years old) situated at three altitudes i.e. Laitkor (Altitude, 2100-2500m msl; Latitude, 25'34"N and Longitude, 91°52"E), Riat Khwan (Altitude, 1200-1700 m msl; Latitude, 25°34"N and Longitude, 91°52"E) and Naya Bunglow (Altitude, 800-1000m msl; Latitude, 25°42"N and Longitude, 91°51"E) were selected (please see Chapter I). Physiographically, the entire area is hilly dominated by pine forests (Pinus kesiya).

Based on the metereological date collected during investigation period the year may be broadly classified into rainy, winter and spring seasons.

Rainy season

The rainy season extends from the middle of April to the middle of October. From the middle of May to July, the highest rainfall could be recorded (Fig. 1.1). Thus rainy season may further be divided into early, middle and late rainy periods.

Winter season

Winter season starts at the end of October and continues upto middle of February. The period is characterised by low temperature and less rainfall. The average maximum and minimum temperatures were recorded

as 16.5 and 2.6°C at Laitkor, 20.0 and 3.05°C at Riat Khwan and 24.3 and 3.4°C at Naya Bunglow respectively. The rainfall was very low.

Spring season

It extends from middle of February upto middle of April which experiences very high wind velocity with less humidity and moderate temperraure.

Irrespective of other parts of the country, the study area does not have a typical summer season. During rainy season (June-August) average maximum and minimum temperatures were 22.2 and 15.8°C at Laitkor, 25.1 and 16.2°C at Riat Khwan and 24.6 and 18.6°C at Naya Bunglow respectively.

The climatological data for the whole period (January-February, 1987 to November-December, 1988) of investigation are presented in Fig.1.1.

The vegetation of the study area can broady be classified into :

i. Subtropical forest
ii. The mixed forest
iii. Temperate forest
iv. Grassland.

The sub-tropical forests are dominated by Pinus kesiya along with some tree species like Alnus nepalensis, Quercus spp., Cedrus deodora, Cryptomaria japonica etc., shrub and herbaceous species like Rhododendron arboreus, Lantana camara, Eupatorium spp. etc.

Mixed sub-tropical forests are confined to restricted areas and are much disturbed. These are dominated by Schima spp., Quercus spp., Alnus nepalensis and a number of rosaceae plants.

Temperate forests are dominated mainly by Quercus griffithii, Myrica esculenta, Betula anoides, Rhododendron arboreum, Photenia notoriam, Daphne spp. etc. and orchids like Dendrobrium spp., Cymbidium spp. etc. Lichens, mosses and ferns on the tree trunks and branches of old trees in moist and humid forests are found.

The grasslands consist of dominant species like Paspalum dilatatum, Pennisetum cladestinum, Imperata cylindrica, Cyperus spp., Arundinella spp., Trifolium repens, Cassia spp. and terrestrial orchids like Spathoglottic pulsesscens, Herminum spp., Arundina spp., Paphiopedilum spp. etc.

The soil of the study area is red lateriate under red loam or brown loam soil type. The texture varies from very light (sand upto 90% in some parts) to very heavy (high clay contents in low lying areas). The soil is rich in nitrogen content in the form of organic matter. However, the amount of phosphorus is very low ranging from .01-.08%. Soil is acidic in reaction and pH varies from 5-6.

Fig. 1.1 : Meteorological data for minimum and maximum temperatures (°C), relative humidity (%) and rainfall (mm) for the period of investigation (Jan.-Feb., 1987-Nov.-Dec., 1988) of the study site situated at three different altitudes (Laitkor 2100-2500m msl, Riat Khwan 1200-1700m msl and Naya Bunglow 800-1000m msl).

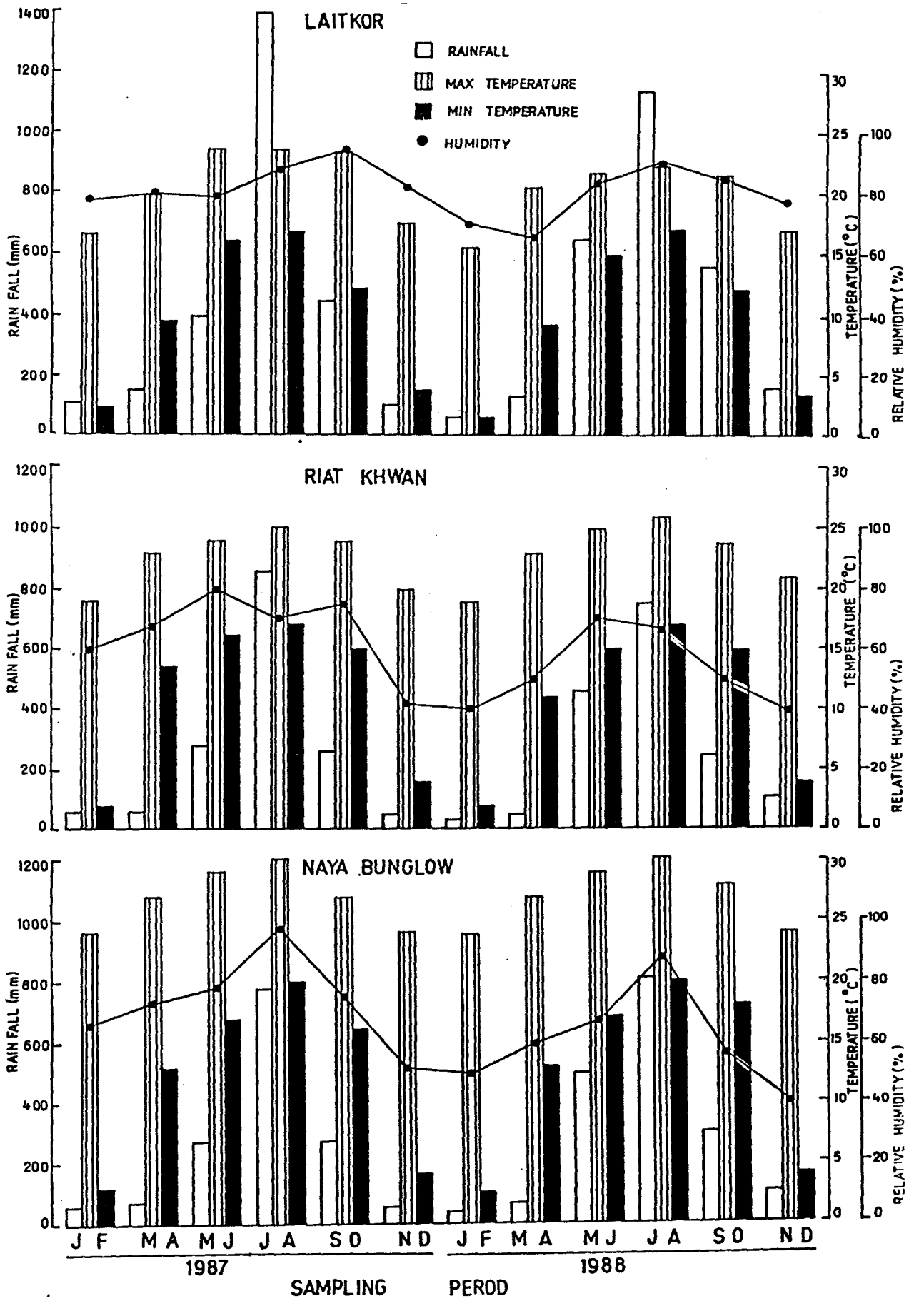

LAITKOR
RAINFALL
MAX TEMPERATURE
MIN TEMPERATURE
HUMIDITY
RAIN FALL (mm)
TEMPERATURE (°C)
RELATIVE HUMIDITY (%)
RIAT KHWAN
NAYA BUNGLOW
J F M A M J J A S O N D J F M A M J J A S O N D
1987
1988
SAMPLING PEROD

Fig. 1.1

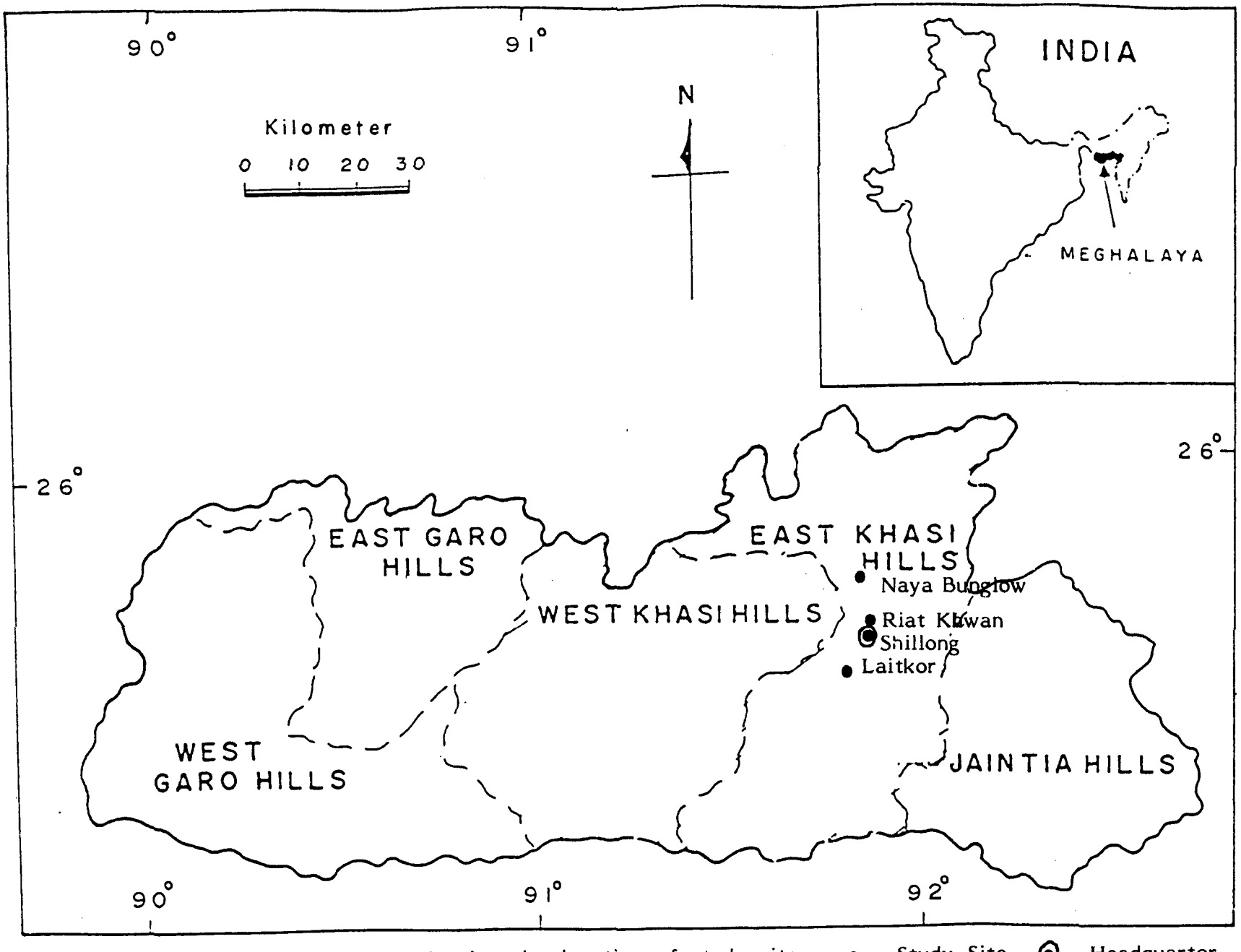


Fig. 1.2 : Map of Meghalaya showing the location of study site. ● - Study Site, ⊙ - Headquarter of East Khasi Hills.

CHAPTER - I

SPATIAL DISTRIBUTION OF ECTOMYCORRHIZAL FUNGI IN PINE STAND AT DIFFERENT ALTITUDES OF KHASI HILLS, MEGHALAYA

Introduction

Occurrence of ectomycorrhizal fungi confirms the inoculum in nature. To study the ecology of mycorrhizal fungi, an information on the distribution of their fruit bodies is essential under natural condition (Last et al., 1981). Some investigations on the spatial and temporal distribution of ectomyorrhizal fungi have been carried out in temperate conditions (Ford et al., 1980; Deacon et al., 1983; Mason et al., 1983; Fleming et al., 1984). Some fungi fruit early with young trees while others appear at later stage of growth of the trees. Based on their appearance these have been categorised as **early stage** and **late stage** fungi respectively (Deacon et al., 1983; Fleming et al., 1984; Fox, 1983). The concept of succession of ectomycorrhizal fungi may provide a basis for selecting them for inoculation in nursery/ afforestation programmes (Mason et al., 1983).

Relationship between the age of the plant and the succession of ectomycorrhizal fungi has been investigated in recent past (Sharma, 1981; Mason et al., 1983), but no attempt has been made to study their distribution at different altitudes in different age groups of pine. Adaptational behaviour

of ectomycorrhizal fungi may differ at various altitudes, probably due to topographical, geographical and environmental conditions.

Variation in the population of ectomycorrhizal fungi may also be regulated by the nutritional status of the soil at different altitudes. Historically, the nutritional response has been most noticeable. Initially ectomycorrhizae were believed to be especially important in the absorption of nitrogen. Frank (1894) had the opinion that mycorrhizal plants absorb nitrogen from nitrate deficient soil but, Hatch (1937), on the basis of his experiments, put forward the view that ectomycorrhiza increases the efficiency of uptake of nutrient especially nitrogen, phosphorus and potassium in short supply. Although, it has been shown that, in general, mycorrhizal fungi have many properties in common (Harley, 1969) but the degree of effectiveness may vary from fungus to fungus at different habitats (Zak, 1973).

Therefore, it may not be possible to generalise an universal pattern of succession for all the ectomycorrhizal fungi at different altitudes. To understand the ecology of ectomycorrhizal fungi it was planned to study their spatial distribution with different age groups of pine stands.

Material and Methods

Selection of site

A list of different pine forests (Pinus kesiya Royle ex. Gordon) in East Khasi Hills, Meghalaya (India) is given in the Appendix Ia (Source: Forest Department, Government of Meghalaya, Shillong). Based on a preliminary survey of these pine woodlands at different altitudes ranging from 800 m (msl) to 2500 m (msl), three stands were selected representing different stages of their growth at each altitude (Table 1.1).

Table 1.1 : Details of the study site at different altitudes

Sites	Altitude	Age of pine stand (years)		
		I	II	III
Laitkor (25°34"N and 91°52'E)	2100-2500 m (msl)	12	7	2
Riat Khwan (25°34"N and 91°52'E)	1200-1700 m (msl)	12	7	2
Naya Bunglow (25°42"N and 91°51'E)	800-1000 m (msl)	12	7	2

Ecology of ectomycorrhizal fungi

(i) Relative abundance

Occurrence and abundance of ectomycorrhizal fungi were determined by collecting their sporocarps from different pinewood lands from January, 1987 to December 1988 at a bimonthly interval. Sporocarps were collected from 60, 25 and 6 m^2 area in 12, 7 and 2 years old pine stands in five replicates. Sporocarps were brought to the laboratory on the same day in sterilized polythelene bags. To ensure the mutual relationship between the sporocarps and roots of pine tree, hyphal connection was traced out from the base of the sporocarps to the mantle of the mycorrhizal roots. Fungal sporocarps were identified with the keys of Chinery (1983), Reid (1969, 1972) and Singer (1962, 1975).

Abundance of the ectomycorrhizal fungi was determined with the formula :

$$\text{Abundance (\%)} = \frac{\text{Total no. of individual spp.}}{\text{Total no. of individuals of all fungal spp.}} \times 100$$

The ectomycorrhizal fungi were grouped in differnt frequency classes, viz. dominant (81-100%), common (61-80%), frequent (41-60%), occasional (21-40%) and rare (1-20%) as suggested by Vittol (1976).

(i) Diversity and evenness

Shannon diversity index for ectomycorrhizal fungi was calculated as suggested by Margalef (1968).

$$\bar{H} = - \sum_{i=1}^{S} (ni/N) \log e (ni/N)$$

where,

$\bar{H}$ = Diversity Index

N = Total no. of individuals of all the species

ni = Total no. of individuals of the individual species.

With the help of values of Diversity Index Evenness of ectomycorrhizal fungi was also calculated as suggested by Pielou (1966).

$$e = \frac{H}{\log e\ S}$$

where,

e = Evenness

H = Diversity Index, and

S = No. of species.

(iii) Succession

Occurrence of ectomycorrhizal fungi was categorised on the basis of their early and late appearance as **early successional** and **late successional** fungi. Ectomycorrhizal fungi which appeared just after the first shower of monsoon were grouped in 'Early Successional' category, whereas those fungi which required more precipitation and appeared late in the seasons

were grouped in the 'Late Successional' category.

'Early and late-stage' fungi were categorised on the basis of their appearance in different ages of pine stands. 'Early-stage' fungi were those fungi which were associated with the young pine trees, whereas, those occurred in old pine stands were termed as 'late-stage' fungi.

(iv) Population of ectomycorrhizae

Population of ectomycorrhizae was observed at bimonthly interval in each selected pine stand. Soil monoliths of 15 x 15 x 15 cm were collected in five replicates with the help of sterilized soil sampler keeping a distance of 100 cm, 50 cm and 25 cm from the tree trunk in 12, 7 and 2 years old pine trees respectively. Soil sample was brought to the laboratory on the same day in a sterilized polythelene container. To separate the mycorrhizae, the soil samples were washed under running tap water in a tray. Mycorrhizae thus separated were collected in a glass dish (15.5 cm diam.) and observed under the sterio binocular microscope and were counted. Total population of mycorrhizae was recorded on per centimeter of the mean root length.

$$\text{Population of ectomycorrhizae/cm} = \frac{\text{Total no. of ectomycorrhizae}}{\text{Total length of short-root (cm)}}$$

Determination of physico-chemical characters of the soil

(i) pH and moisture content

pH of the soil samples was determined with the help of digital pH meter. Ten grams fresh soil was suspended in 50 ml double distilled water and stirred for 15 minutes before taking the pH.

Moisture content of the soil was determined by drying 10 g freshly collected soil in a hot air oven at 105°C for 24 hrs. After 24 hrs, the dried

soil was cooled at room temperature in a desiccator and weighed. Percentage moisture content was calculated as follows :

$$\text{Moisture content (\%)} = \frac{\text{Loss of wt. on drying (g)}}{\text{Initial sample weight (g)}} \times 100$$

(ii) Soil temperature

Soil temperature was measured with the help of soil thermometer in degree celcius. Soil thermometer was bored into the soil for five minutes and the temperature was noted down. Six replicates of soil temperature were measured.

(iii) Organic-carbon

Walkley and Black's (1934) rapid titration method was followed for determination of organic carbon. To 0.5 g of sieved soil (through 0.2 mm mesh) in a 500 ml conical flask and 10 ml of $K_2Cr_2O_7$ (IN) and 20 ml of H_2SO_4 were added and left for 30 minutes. The mixture was then diluted with 200 ml double distilled water. Finally, it was titrated with $FeSO_4$ (IN) using diphenylamine as an indicator. The percentage organic carbon was calculated according to the following formula :

$$\text{Organic carbon (\%)} = \frac{V_1 - V_2}{W} \times 0.003 \times 100$$

where,

V_1 = volume of K_2Cr_2O7

V_2 = volume of $FeSO_4$ and

W = weight of soil (g).

(iv) Total nitrogen

Kjeldahl's digestion method was followed for the determination of total nitrogen (Jackson, 1973). To 0.5 g sieved soil (sieved through 0.2 mm mesh) in a 100 ml Kjeldahl flask, 2 g potassium sulphate-mercuric oxide mixture (20:1) and 3 ml of H_2SO_4 were added. The digestion was carried out on a digestion unit. At the end of digestion, when the colour of the solution turned transparent, the heating was stopped and the flasks were allowed to cool. The content was diluted with 50 ml distilled water. The solution was then ready for the determination of ammonium nitrogen by spectrophotometer.

To 2 ml digested solution in a 50 ml volumetric flask, 8 ml alkaline Rochille reagent (60 grams of sodium-potassium tartrate in 600 ml distilled water) was added and mixed. After that 1 ml sodium nitroprusside solution (0.16%, W/v) was added and mixed. Now 2 ml solution of sodium phenol reagent (50 g phenol was dissolved in 250 ml 40% NaOH and diluted to 400 ml with distilled water) was added and mixed. Finally 1 ml sodium hypochlorite reagent (1N) was added and the volume of the solution in the volumetric flask was made to 50 ml adding distilled water and mixed well. The flasks were then kept on a water bath at 40°C for 20 minutes to develop blue colour of the solution. Later on, the flasks were allowed to cool and the optical density was measured at 625 nm against blank. A standard curve was prepared using NH_4+ nitrogen and with the help of the standard curve percentage of total nitrogen (ammonium) was calculated as follows :

$$\text{Nitrogen (\%)} = \frac{\text{C(mg) x solution volume (ml)}}{\text{10 x aliquot (ml) x sample wt. (g)}}$$

where,

C = mg NH_4 + nitrogen obtained from the standard curve.

(v) Available phosphorus

To determine the available phosphorus in soil the method of Jackson (1962) outlined by Misra (1968) was followed. To 5 g of sieved soil (sieved through 0.2 mm mesh) in a 500 ml conical flask 200 ml of 0.002N H_2SO_4 was added and was shaken for 30 minutes. The suspension was filtered through a Whatman No.42 filter paper. 20 ml aliquot was transferred into a 50 ml volumetric flask and added with 2-3 drops of dinitrophenol indicator (0.25% 2,4 dinitrophenol indicator). The solution became yellow. Now 2N H_2SO_4 was added drop by drop till the solution turned colourless and then the solution was diluted and the volume was made 45-47 ml. After that 2 ml of sulphomolybdic acid solution was added and the flask was shaken. Finally, 0.5 ml solution of chloro-stanous acid was added and was shaken well. After 5 minutes, the colour of the solution turned blue and the percent transmission was read at 660 nm against blank. A calibration curve was prepared. With the help of the curve phosphorus in ppm in the test solution was calculated. Available phosphorus was calculated as follows :

$$\text{Phosphorus in the soil (ppm)} = \frac{\text{ppm P in the solution x extraction solution (ml)}}{\text{wt. of soil (g)}}$$

When 5 g of soil has been extracted with 200 ml solution, ppm phosphorus in soil = ppm phosphorus in solution x 200.

(vi) Exchangeable potassium

Potassium was extracted in ammonium acetate solution (pH-7) which was prepared by mixing 575 ml of glacial acetic acid with 600 ml of ammonium solution and diluted to 10 litres with distilled water. The pH was adjusted to 7 $\pm$ 0.05 with the help of acetic acid and ammonium solutions.

To 5 g of sieved soil (sieved through 0.2 mm sieve), 125 ml of extraction solution was added. It was allowed for constant stirring for one hour and then filtered through Whatman No.44 filter paper. The exchangeable potassium was read in a flame photometer (Allen, 1974) and converted into known unit through standard graph. The percentage potassioum was calculated as follows :

$$\text{Potassium (\%)} = \frac{\text{C(ppm) x solution volume (ml)}}{10^4 \text{ x sample weight (g)}}$$

Where,

C = ppm potassium in the solution obtained by the standard graph.

Collection of weather data of the study site

(i) Total precipitation

Precipitation was measured with the help of rain guages fitted at all the study sites prior to first shower of monsoon. Regular observation was made and rainfall was recorded in millimeters on each sampling date.

(ii) Percentage relative humidity

Relative humidity was measured with the help of hygrometer at each study site.

(iii) Atmospheric temperature

Atmospheric temperature was measured with the help of ordinary thermometer in degree celcius in each pine stand.

Results

Eleven ectomycorrhizal fungi were found in the first year and fourteen in second year of investigation (Appendix Ib). Few of them were observed

for short duration while others survived for a longer period (Fig. 1.3).

Boletus sp., Lactarius sp., Tricholoma sp., Russula sp., Amanita sp. and Gomphidium sp. were found at the higher altitude. In the first year of investigation Boletus sp. and Lactarius sp. occurred as an early successional species in 12 years old stand but in the second year Boletus sp. appeared in the months of July and August. Amanita sp. was absent in all the stands in first year of the study, which was an early colonizing species along with Lactarius sp. and Russula sp. Gomphidium sp., Tricholoma sp. and Russula sp. were found as late successional species. Boletus sp. indicated its broad ecological amplitude by occurring from May-December (Fig. 1.3). Sporocarps of higher fungi in 2 and 7 year old stands were absent in the first year (Fig. 1.4) whereas, in the following year, Boletus sp. and Lactarius sp. were observed. In 7 years old stand, Boletus sp. was noticed in the previous year, while the Lactarius sp. and Russula sp. were reported in the successive year. Boletus sp. was dominant genus during the last phase of its occurrence at the higher altitude in 12 and 7 years old pine stands in the previous year (Table 1.2). In the successive year it also occurred dominantly in 2 years old pine stand in the last phase of its occurrence (Table 1.2).

At the middle altitude, Boletus sp. was dominant in the previous year in 12 and 7 years old stands. Suillus sp., Tricholoma sp., Lactarius sp. and Hygrophorus sp. have been observed in 12 years old pine stand in both the years, while Collybia sp. occurred only in the previous year and Cortinarius sp. and Scleroderma sp. in the next year (Table 1.2). Boletus sp. and Lactarius sp. were early colonizing species in 12 years old pine stand. In 7 years old stand, Lactarius sp. was late colonizing species. Suillus sp., Tricholoma sp. and Hygrophorus sp. were reported in July and August in

12 years old stand, but Suillus sp. could survive upto October. In this stand also, Boletus sp. showed a broad ecological amplitude followed by Suillus sp. (Fig. 1.3). Collybia sp. was found with 12 years old stand only in first year of the study. Pisolithus sp. was rare in July-October in 7 years old stand. Lactarius sp. was recorded during July-August. Boletus sp. was noticed from May-December. Suillus sp. was found during July-August in first year and during May-December in second year. In 7 years old stand Russula sp. was absent in the previous year, but was noticed during July-October in the second year. No sporocarp was observed in 2 years old pine stand.

The population of Scleroderma sp. was highest in the 12 years old stands at the lower altitude. It occurred from May-December and was the dominant fungus in old stand. Though, the population of Boletus sp. was less than Scleroderma sp. but it produced the sporocarps from May-December. In the first year of study, Lactarius sp. was noticed during May-August with highest abundance, but in second year it appeared for a shorter duration with a steep fall in population. Suillus sp. was found as secondary colonizing species in first and second year of study in old pine forest respectively. Hygrophorus sp., Russula sp. and Cortinarius sp. were early colonizing species in second year. Hygrophorus sp. and Russula sp. were found upto October while Cortinarius sp. occurred upto August. Tricholoma sp. appeared only in the second year during July-August. Tricholoma sp. also appeared only in the second year during July-August. Seven years old plantation harboured maximum population of Boletus sp. followed by Amanita sp. Suillus sp. was found during July-October and May-October in first and second years respectively. Elaphomyces sp., Clitocybe sp., Russula sp. and Terfezia sp. were occasionally noticed in 7 years old plantation.

No sporocarp was found in 2 years old stand in first year of study but in the second year, Boletus sp. was reported during July-August.

Maximum ectomycorrhizal fungi were found at the lower altitude and least at the higher altitude (Fig. 1.4). Ectomycorrhizal fungi were inversely proportional to the altitude of the pine stands.

In the 12 years old plantation, Boletus sp., Lactarius sp., and Tricholoma sp. were observed at all the altitudes but Gomphidium sp. was specific only at the higher altitude (Fig. 1.3). Suillus sp., Hygrophorus sp. and Cortinarius sp. were common at two other altitudes, but were absent from the higher altitude (Fig. 1.3).

In the 7 years old stand, Boletus sp. and Russula sp. were common at all the altitudes. Lactarius sp. was collected only from the middle and higher altitude while Suillus sp. was common at lower altitudes. Pisolithus sp. was reported from the middle altitude only, while Elaphomyces sp., Amanita solitaria, Clitocybe sp. and Terfezia sp. were specific to lower altitude. However, Amanita sp. was only recorded from the higher altitude in 12 years old pine stand. In 2 years old plantation few sporocarps were observed from higher and lower altitudes. Boletus sp. was common at both altitudes while Lactarius sp. was confined to the higher altitude. No sporocarp was reported from the middle altitude associated with 2 years old stand.

Diversity of ectomycorrhizal fungi was directly proportional to the age of the pine stand (Table 1.3). Evenness of the ectomycorrhizal fungi also increased with the increase of the age of pine stand (Table 1.4).

Variation in composition and population of "Early-stage" and "Late-stage" fungi was observed at different altitudes. Some genera of "Early-

stage" and "Late-stage" fungi were common at different altitudes (Table 1.5). Similar result was obtained with the "Early-successional" and "Late-successional" fungi (Table 1.6).

Population of ectomycorrhizae at different altitudes and in different age groups of pine stands also followed the same pattern as of ectomycorrhizal fungi (Fig. 1.5).

Population of fungi was observed inversely proportional to the altitude and directly proportional to the age groups of pine.

Minimum population of ectomycorrhizal fungi was recorded in younger pine stand and maximum population in the older pine stand (Fig. 1.4).

Population of mycorrhizae was directly proportional to the age of pines. Population of ectomycorrhizae increased significantly as the age of pine increased (Table 1.12 and 1.13). It was found more in all the ages of pine in the second year of investigation than first year of study (Fig. 1.5).

Physico-chemical properties of the soil

Temperature and moisture content of the soil were minimum during winter season (December-February) and maximum during rainy season (June-August). Positive correlation between the population of mycorrhizae and soil temperature and moisture content was observed (Figs. 1.16-1.21); except in the case of 2 years old pine stand at upper and lower altitudes where the correlation between soil temperature and ectomycorrhizal population was negative in the previous year (Fig. 1.18). In the same year negative correlation was observed between soil temperature and the population of ectomycorrhizae in 7 years old pine stand at lower altitude (Fig. 1.17). The population of the fungi increased in July-August and decreased there-

after (Fig. 1.4). Maximum temperature of soil was observed during May-August (Fig. 1.14), while moisture content had its peak during July-August (Fig. 1.15).

The study site at highest altitude experienced highest soil moisture compared to lower sites (Fig. 1.15) which was inversely proportional to the population of fungi while, the population of fungi was directly proportional to the soil temperature.

No significant variation in soil pH was observed throughout the investigation period. The pH was observed between 4-6 (Table 1.7). pH of the soil did not affect the population of fungi and the mycorrhizae.

Organic carbon of the soil was high during winter season and was less during rainy season (Table 1.8). Organic carbon has been found inversely proportional to the fungal population and the ectomycorrhizae.

Total nitrogen (Ammonium nitrogen) content of the soil increased considerably during spring and rainy seasons and decreased in winter (Table 1.9). Soil nitrogen was found directly proportional to the ectomycorrhizae and fungal population.

Available phosphorus in soil was recorded minimum at the highest altitude compared to lower altitudes (Table 1.10) and was directly related to the less number of mycorrhizae and their population at the highest altitude. Peak of the phosphorus content was obtained during May-June.

Exchangeable potassium in the soil was less affected by the altitudes. The potassium peak was obtained during July-August (Table 1.11).

Climatic condition of the study site

Atmospheric temperature and relative humdity were found to increase

with the increase in precipitation. Temperature, relative humidity and precipitation were found directly proportional to the ectomycorrhizae and their sporocarp population. Peak of temperature (Fig. 1.6), relative humidity (Fig. 1.7) and rainfall (Fig. 1.1) was obtained during July-August which coincided with the peak of population of fungi and ectomycorrhizae. Variation in temperature, relative humdity and precipitation was obtained at different altitudes. Maximum rainfall and relative humidity and minimum temperature were recorded at higher altitude compared to lower altitudes. Mycorrhizae and their sporocarps were directly porportional to the temperature while inversely proportional to the rainfall and relative humidity.

Postive correlations have been found between the population of ectomycorrhizae and the climatic factors (Fig. 1.8-1.13).

Population of ectomycorrhizae in 12 years old pine plantation at all three altitudes was found positively correlated to the atmospheric temperature, relative humdity and rainfall in 1987 (Fig. 1.8) but in 1988 a negative correlation was found with relative humdity at the higher altitude (Fig. 1.11).

In 7 years old pine stand positive correlations were found with temperature, relative humidity and rainfall at all the three altitudes in both the years (Fig. 1.9 and 1.12), but in 1988 mycorrhizal population was found negatively correlated with relative humidity at the middle altitude (Fig. 1.12).

In 2 years old stand mycorrhizal population was found positively correlated with the relative humidity in 1987 (Fig. 1.10) but a negative correlation was obtained at the lower altitude in 1988 (Fig. 1.13). Positive correlations were also observed with rainfall and temperature in 1988 (Fig. 1.1[illegible]), however, in the previous year negative correlation was found with rainfall at the middle altitude and with the temperature at the lower altitude

(Fig. 1.10).

Discussion

Inverse correlationship between the population of ectomycorrhizal fungi and the altitudes of pine stands was observed. Similar pattern has been reported by Sharma (1981) about the correlation between the population of ectomycorrhizae and the environmental conditions.

The study site at higher altitude received maximum precipitation compared to lower altitudes, which might have leached out the nutrients from the soils and washed out the mycorrhizal propagules alongwith other micro-organisms resulting in the low population of sporocarpic fungi (Deka et al., 1989). The growth of ectomycorrhizal fungi has been reported to be reduced by either antibiotics producing bacteria, or competiting for the energy source under rainfed condition (Bowen and Theodorou, 1979).

Soil moisture was also inversely proportional to the population of ectomycorrhizae and the fungal sporocarps. High water content of the soil, than optimum level which favours the microbial growth, might have reduced the concentration of inorganic and organic nutrients and root exudates resulting in a decrease in their availability to the fungi. High water content and low soil temperature at the higher altitude might have made the conditions unfavourable for the growth of ectomycorrhizal fungi. Theodorou (1978) observed the maximum mycorrhizal infection at moderate water contents of soil (13%-20%) and a decrease in the number of fungi at higher moisture level (27%).

Soil and air temperatures were directly proportional to the abundance of the ectomycorrhizal fungi as well as mycorrhizae, which increased with the increase in temperature and decreased with the decrease in temperature.

Temperature was inversely porportional to the altitudes. Low temperature may reduce the metabolic rate of fungi and their interaction with the microbes which may reduce the rate of release of the root exudates from the host root as indicated by Theodorou and Bowen (1917) limiting the energy source to the heterotrophic population and reducing the colonization. Theodorou and Bowen (1971) have reported reduction in colonization by some strains of R. luteolus when reducing the soil temperature from 20°C to 15°C. Poor growth of ectomycorrhizal fungi at low temperature has been reported by Cline et al. (1987), however, some fungi have been reported to grow at low temperatures (Moser, 1958).

Age of pines was directly proportional to the population of mycorrhizal fungi as well as to the mycorrhizae. The older pine stand experienced more litter compared to younger pine stand. Accumulation of litter on the soil surface can result in increased humus in sub-tropical conditions which harboured more microorganisms (Tan and Nopamornbodi, 1979). Burges and Latter (1960) and Prat (1960) were of the opinion that humic acid serves as a source of energy for mnicroorganisms. More humus in the older stand than younger stand could be directly correlated with the high population of ectomycorrhizal fungi.

The older trees of pine may release greater amount of root exudates than the younger trees. The quantitative and qualitative changes in root exudates have been assigned to the age of plant which may influence the population of microbes in rhizosphere (Rovira, 1969). Root exudates are known to promote the growth of ectomycorrhizal fungi (Melin, 1963; Bowen and Theodorou, 1973; Fries et al., 1985). They can also stimulate spore germination of ectomycorrhizal fungi (Fries and Birraux, 1980; Birraux and Fries, 1981; Fries, 1981). Increased number of mycorrhizae in old stands

of pine could decompose and grow readily on sterilized litter. The less number of fungi during winter could mainly be assigned to low temperature inspite of increased amount of organic carbon of the soil. On the other hand high microbial activity and population of ectomycorrhizal fungi during rainy season might have rendered low level of organic carbon.

Phosphorus is readily absorbed by the ectomycorrhizal fungi unavailable to the higher plants (Bowen, 1973). During rainy season high phosphorus content of the soil was correlated to the population of the ectomycorrhizal fungi. Harley (1969) had shown that low temperature and metabolic inhibitors inhibit phosphate uptake by the ectomycorrhizal fungi. The reason for less population of ectomycorrhizal fungi at higher altitude could be attributed to low temperature compared to lower altitudes. Potassium uptake by the ectomycorrhizal fungi is also affected by the temperature and metabolic activity (Harley and Smith, 1983). Low temperature at the higher altitude might have affected the absorption of potassium adversely and hence the ectomycorrhizae.

Studies on the ecology of ectomycorrhizae suggested that certain ectomycorrhizal fungi like Pisolithus tinctorius, Boletus sp., Suillus sp., Lactarius sp., Elaphomyces sp., Amanita sp., Clitocybe sp. and Terfezia sp. can be exploited in the afforestation programme in the North-Eastern region due to their colonizing habit with the young pine trees.

Table 1.2 : Abundance (%) of ectomycorrhizal fungi in different age groups of pine stands at different altitudes

Ectomycorrhizal fungi	Yr. of sampling	Liatkor (2100-2500m msl)												Riat Khwan (1200-1700m msl)												Naya Bunglow (800-1000m msl)											
		12 Yrs.				7 Yrs.				2 Yrs.				12 Yrs.				7 Y s.				2 Yrs				12 Yrs.				7 Yrs.				2 Yrs.			
		Sampling period																																			
		J	A	O	D	J	A	O	D	J	A	O	D	J	A	O	D	J	A	O	D	J	A	O	D	J	A	O	D	J	A	O	D	J	A	O	D
Amanita sp.	1987																													29	17						
	1988	18																												24	37						
Boletus sp.	1987	70	36	60	100		100	100						29	16	63	100	100	2	50	100					20	21	37	45	100	50	72	100				
	1988		7	37	100					78	63	100		67	8	34	100	100	21	36	75						9	23	39	25	4	64	100	100	100		
Clitocybe sp.	1987																																				
	1988																													9	17						
Collybia sp.	1987														3																						
	1988																																				
Cortinarius sp.	1987																																				
	1988														51											35	39										
Elaphomyces sp.	1987																														10						
	1988																													9	11						
Gomphidium sp	1987		7	14																																	
	1988		5																																		
Hygrophorus sp.	1987														3																						
	1988														5											8	5	8									
Lactarius sp.	1987	30	21											73	59				3							57	49										
	1988	73	57			70	83			22	37			33	12				40								5										
Pisolithus sp.	1987																		16	50																	
	1988																		17	25																	
Russala sp.	1987																																				
	1988	9	5	25		30	17	100											10	18						19	6	12		15	11	14					
Scleroderma sp.	1987																									23	25	56	55								
	1988													7	33											38	22	50	46								
Suillus sp.	1987													9	37				11								6	4			11	11					
	1988													9	33				12	18	25						6	8	15	12	20	22					
Terfezia sp.	1987																																				
	1988																													6							
Tricholoma sp.	1987		14	40										11																							
	1988		9	38										5												7											

J = May-June; A = July-August; O = September-October; D = November-December.

Table 1.3 : Diversity index of ectomycorrhizal fungi at different altitudes in different pine stands

Altitude	Age of pine stands (years)	Sampling period (season)					
		Spring		Rainy		Winter	
		1987	1988	1987	1988	1987	1988
Laikor (2100-2500 m msl)	12	0	0	1.21	1.52	0	0
	7	0	0	0	.51	0	0
	2	0	0	0	.43	0	0
Riat Khwan (1200-1700 m msl)	12	0	0	1.20	1.58	0	0
	7	0	0	.90	1.35	0	.81
	2	0	0	0	0	0	0
Naya Bunglow (800-1000 m msl)	12	0	0	1.44	2.10	.99	1.25
	7	0	0	.97	1.79	0	0
	2	0	0	0	0	0	0

Table 1.4 : Evenness of ectomycorrhizal fungi at different altitudes in different pinus stands

Altitude	Age of pine stands (years)	Sampling period (season) Spring 1987	Spring 1988	Rainy 1987	Rainy 1988	Winter 1987	Winter 1988
Laikor (2100-2500 m msl)	12	0	0	.91	1.80	0	0
	7	0	0	0	.51	0	0
	2	0	0	0	.43	0	0
Riat Khwan (1200-1700 m msl)	12	0	0	.82	.90	0	0
	7	0	0	.62	.65	0	.81
	2	0	0	0	0	0	0
Naya Bunglow (800-1000 m msl)	12	0	0	.79	.85	.99	.79
	7	0	0	.46	.77	0	0
	2	0	0	0	0	0	0

Table 1.5 : 'Early-Stage' and 'Late-Stage' Ectomycorrhizal fungi at different altitudes

Altitudes	Ectomycorrhizal fungi			
	Early Stage		Late Stage	
	1987	1988	1987	1988
Laitkor (2100-2500 m msl)	Boletus spp.	Boletus spp. Lactarius spp. Russula spp.	Tricholoma spp. Gomphidium spp.	Amanita spp. Tricholoma spp. Gomphidium spp.
Riat Khwan (1200-1700 m msl)	Boletus spp. Pisolithus tinctorius Suillus spp. Lactarius spp.	Boletus spp. Pisolithus tinctorius Russula spp. Lactarius spp. Suillus spp.	Hygrophorus spp. Collybia spp. Tricholoma spp.	Hygrophorus spp. Tricholoma spp. Scleroderma sp
Naya Bunglow (800-1000 m msl)	Boletus spp. Amanita spp. Suillus spp. Elaphomyces spp.	Boletus spp. Amanita spp. Russula spp. Clitocybe spp. Suillus spp. Elaphomyces spp. Terfezia spp.	Scleroderma spp. Lactarius spp. Tricholoma spp. Cortinarius spp.	Scleroderma spp. Cortinarius spp. Hygrophorus spp. Tricholoma spp. Lactarius spp.

Table 1.6 : 'Early-Successional' and 'Late-Successional' ectomycorrhizal fungi at different altitudes

Altitudes	Ectomycorrhizal fungi			
	Early Successional		Late Successional	
	1987	1988	1987	1988
Laitkor (2100-2500 m msl)	Boletus spp. Lactarius spp.	Boletus spp. Lactarius spp. Russula spp. Amanita spp.	Gomphidium spp. Tricholoma spp.	Gomphidium spp. Tricholoma spp.
Riat Khwan (1200-1700 m msl)	Boletus spp. Lactarius spp.	Boletus spp. Lactarius spp.	Hygrophorus spp. Collybia spp. Tricholoma spp. Suillus spp. Pisolithus spp.	Hygrophorus spp. Tricholoma spp. Suillus spp. Pisolithus spp. Scleroderma spp. Russula spp.
Naya Bunglow (800-1000 m msl)	Boletus spp. Lactarius spp. Scleroderma spp.	Boletus spp. Scleroderma spp. Russula spp. Cortinarius spp. Hygrophorus spp. Clitocybe spp. Amanita spp. Elaphomyces spp. Terfezia spp.	Tricholoma spp. Suillus spp. Cortinarius spp. Elaphomyces spp. Amanita spp.	Tricholoma spp. Lactarius spp.

Table 1.7 : Soil pH at different altitude in different age of pine stand

Site Altitude	Year of sampling	Jan.-Feb.			Mar.-Apr.			May-June			Jul.-Aug.			Sept.-Oct.			Nov.-Dec.		
		Age of pine stand (years)																	
		12	7	2	12	7	2	12	7	2	12	7	2	12	7	2	12	7	2
Laitkor (2100-2500m msl)	1987	5.57	5.60	5.72	5.52	4.42	5.54	5.29	5.32	5.61	5.26	5.24	5.53	5.53	5.69	5.79	5.55	5.72	5.68
	1988	5.30	5.23	5.12	5.52	4.42	5.54	5.12	5.06	5.27	5.30	5.26	5.50	5.79	5.80	5.62	5.68	5.68	5.55
Riat Khwan (1200-1700m msl)	1987	5.74	5.67	5.64	5.90	5.85	5.68	5.72	5.55	5.53	5.28	5.29	5.52	5.84	5.67	5.86	5.81	5.74	5.79
	1988	5.05	5.30	5.24	5.90	5.85	5.68	5.55	5.45	5.46	5.28	5.31	5.68	5.82	5.55	5.51	5.65	5.61	5.65
Naya Bunglow (800-1000m msl)	1987	5.54	5.49	5.61	5.18	5.06	5.34	5.38	5.48	5.45	5.17	5.20	5.24	5.50	5.65	5.34	5.75	5.51	5.79
	1988	5.30	5.25	5.40	5.18	5.06	5.34	5.29	5.35	5.36	5.22	5.26	5.40	5.62	5.46	5.52	5.49	5.56	5.65

Table 1.8 : Organic carbon (%) of the soil of different ages of pine stands at different altitudes

Site (Altitude)	Year of sampling	Jan.-Feb.			Mar.-Apr.			May-June			Jul.-Aug.			Sept.-Oct.			Nov.-Dec.		
		Age of pine stand (years)																	
		12	7	2	12	7	2	12	7	2	12	7	2	12	7	2	12	7	2
Laitkor (2100-2500m msl)	1987	4.1	2.8	3.9	3.7	4.0	3.8	4.1	4.3	4.4	3.2	2.9	2.9	5.5	5.2	5.5	4.2	4.4	4.9
	1988	3.7	2.6	3.2	2.9	4.4	3.5	4.1	3.3	3.7	3.0	3.4	2.9	5.4	5.1	5.5	4.1	4.2	4.7
Riat Khwan (1200-1700m msl)	1987	0.8	1.7	0.7	1.6	1.6	1.7	1.0	2.5	1.5	1.1	1.0	1.4	2.5	2.8	2.3	1.0	1.6	1.9
	1988	1.0	1.6	0.6	1.4	1.2	1.6	0.4	1.4	1.2	0.9	0.5	1.2	2.4	2.6	2.2	1.0	1.5	2.1
Naya Bunglow (800-1000m msl)	1987	0.7	1.3	1.4	1.2	1.1	0.9	0.8	0.6	1.5	1.1	0.8	0.9	1.9	2.1	1.4	1.4	1.3	0.4
	1988	0.8	0.9	1.6	1.0	1.0	0.8	0.5	0.2	1.0	1.2	1.0	1.0	2.0	2.2	1.7	1.3	1.3	0.9

Table 1.9 : Total nitrogen content (%) of the soil of different ages of pine stands at different altitudes

Site (Altitude)	Year of sampling	Jan.-Feb.			Mar.-Apr.			May-June			Jul.-Aug.			Sept.-Oct.			Nov.-Dec.		
		Age of pine stand (years)																	
		12	7	2	12	7	2	12	7	2	12	7	2	12	7	2	12	7	2
Laitkor (2100-2500m msl)	1987	0.12	0.14	0.13	0.36	0.50	0.35	0.41	0.26	0.25	0.56	0.36	0.34	0.31	0.25	0.26	0.20	0.17	0.21
	1988	0.16	0.14	0.15	0.44	0.45	0.51	0.45	0.56	0.36	0.45	0.34	0.41	0.36	0.27	0.26	0.22	0.21	0.18
Riat Khwan (1200-1700m msl)	1987	0.09	0.12	0.10	0.16	0.17	0.19	0.15	0.19	0.14	0.14	0.24	0.16	0.13	0.16	0.15	0.10	0.15	0.17
	1988	0.12	0.13	0.14	0.18	0.19	0.21	0.16	0.22	0.17	0.15	0.19	0.22	0.16	0.16	0.18	0.11	0.12	0.13
Naya Bunglow (800-1000m msl)	1987	0.8	0.10	0.09	0.19	0.18	0.23	0.12	0.14	0.21	0.15	0.14	0.15	0.16	0.15	0.18	0.16	0.16	0.08
	1988	0.11	0.09	0.09	0.21	0.22	0.25	0.23	0.21	0.20	0.16	0.18	0.19	0.17	0.19	0.20	0.17	0.14	0.15

Table 1.10 : Available phosphorus (%) content of the soil of different ages of pine stands at different altitudes

Site (Altitude)	Year of sampling	Jan.-Feb.			Mar.-Apr.			May-June			Jul.-Aug.			Sept.-Oct.			Nov.-Dec.		
		Age of pine stand (years)																	
		12	7	2	12	7	2	12	7	2	12	7	2	12	7	2	12	7	2
Laitkor (2100-2500m msl)	1987	0.02	0.03	0.02	0.03	0.02	0.02	0.04	0.04	0.03	0.03	0.04	0.03	0.03	0.04	0.04	0.03	0.03	0.04
	1988	0.01	0.02	0.02	0.02	0.03	0.03	0.04	0.05	0.03	0.03	0.04	0.03	0.04	0.04	0.03	0.02	0.03	0.03
Riat Khwan (1200-1700m msl)	1987	0.05	0.06	0.05	0.06	0.05	0.06	0.08	0.08	0.07	0.07	0.08	0.06	0.06	0.07	0.08	0.06	0.07	0.06
	1988	0.04	0.04	0.06	0.05	0.06	0.06	0.08	0.08	0.07	0.06	0.07	0.07	0.06	0.07	0.06	0.06	0.07	0.07
Naya Bunglow (800-1000m msl)	1987	0.04	0.06	0.05	0.05	0.06	0.06	0.08	0.08	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.06	0.08	0.08
	1988	0.05	0.06	0.05	0.05	0.05	0.06	0.07	0.08	0.07	0.06	0.06	0.06	0.07	0.06	0.07	0.07	0.08	0.07

Table 1.11 : Exchangeable potassium (%) content of the soil of different ages of pine stands at different altitudes

Site (Altitude)	Year of sampling	Jan.-Feb.			Mar.-Apr.			May-June			Jul.-Aug.			Sept.-Oct.			Nov.-Dec.		
		Age of pine stand (years)																	
		12	7	2	12	7	2	12	7	2	12	7	2	12	7	2	12	7	2
Laitkor (2100-2500m msl)	1987	0.17	0.16	0.16	0.15	0.16	0.16	0.20	0.18	0.19	0.20	0.22	0.21	0.18	0.15	0.17	0.13	0.15	0.15
	1988	0.16	0.14	0.15	0.20	0.17	0.18	0.22	0.21	0.22	0.23	0.24	0.20	0.17	0.14	0.18	0.14	0.15	0.12
Riat Khwan (1200-1700m msl)	1987	0.18	0.17	0.17	0.21	0.22	0.20	0.32	0.23	0.33	0.34	0.45	0.32	0.46	0.45	0.35	0.23	0.31	0.22
	1988	0.22	0.18	0.19	0.22	0.22	0.32	0.42	0.25	0.24	0.42	0.44	0.31	0.45	0.32	0.28	0.27	0.23	0.24
Naya Bunglow (800-1000m msl)	1987	0.21	0.16	0.17	0.2	0.25	0.18	0.35	0.32	0.42	0.55	0.35	0.32	0.22	0.27	0.31	0.19	0.21	0.22
	1988	0.21	0.22	0.22	0.31	0.34	0.19	0.37	0.32	0.47	0.52	0.44	0.47	0.35	0.32	0.26	0.18	0.26	0.22

Table 1.12: Analysis of variance for population of ectomycorrhizae at different altitudes in 1988

Source of variance	SS	df	MSS	Calculated value	F value at 5%
Effect of altitude	1163.70	2	582.85	127.1*	3.49
Effect of sampling period	2967.65	5	593.53	129.5*	2.71
Effect of age	8102.71	2	4051.35	883.7*	3.49
Effect of altitude x sampling period	60.09	10	6.00	1.3	2.37
Effect of altitude x age	120.52	4	30.13	6.6*	2.87
Effect of age x sampling period	1270.42	10	127.04	27.7*	2.37
Error	91.69	20	4.58	0	-
Total	13778.78	53	259.98	0	-

* Significant at P = 0.05

Table 1.13: Analysis of variance for population of ectomycorrhizae at different altitudes in 1987

Source of variance	SS	df	MSS	Calculated value	F value at 5%
Effect of altitude	404.48	2	202.24	31.1*	3.49
Effect of sampling period	1545.92	5	309.18	47.5*	2.71
Effect of age	2716.26	2	1358.13	208.7*	3.49
Effect of altitude x sampling period	98.18	10	9.81	1.5	2.37
Effect of altitude x age	43.84	4	10.96	1.7	2.87
Effect of age x sampling period	613.07	10	61.31	9.4*	2.37
Error	130.16	20	6.51	0	-
Total	5551.93	53	104.75	0	-

* Significant at P = 0.05

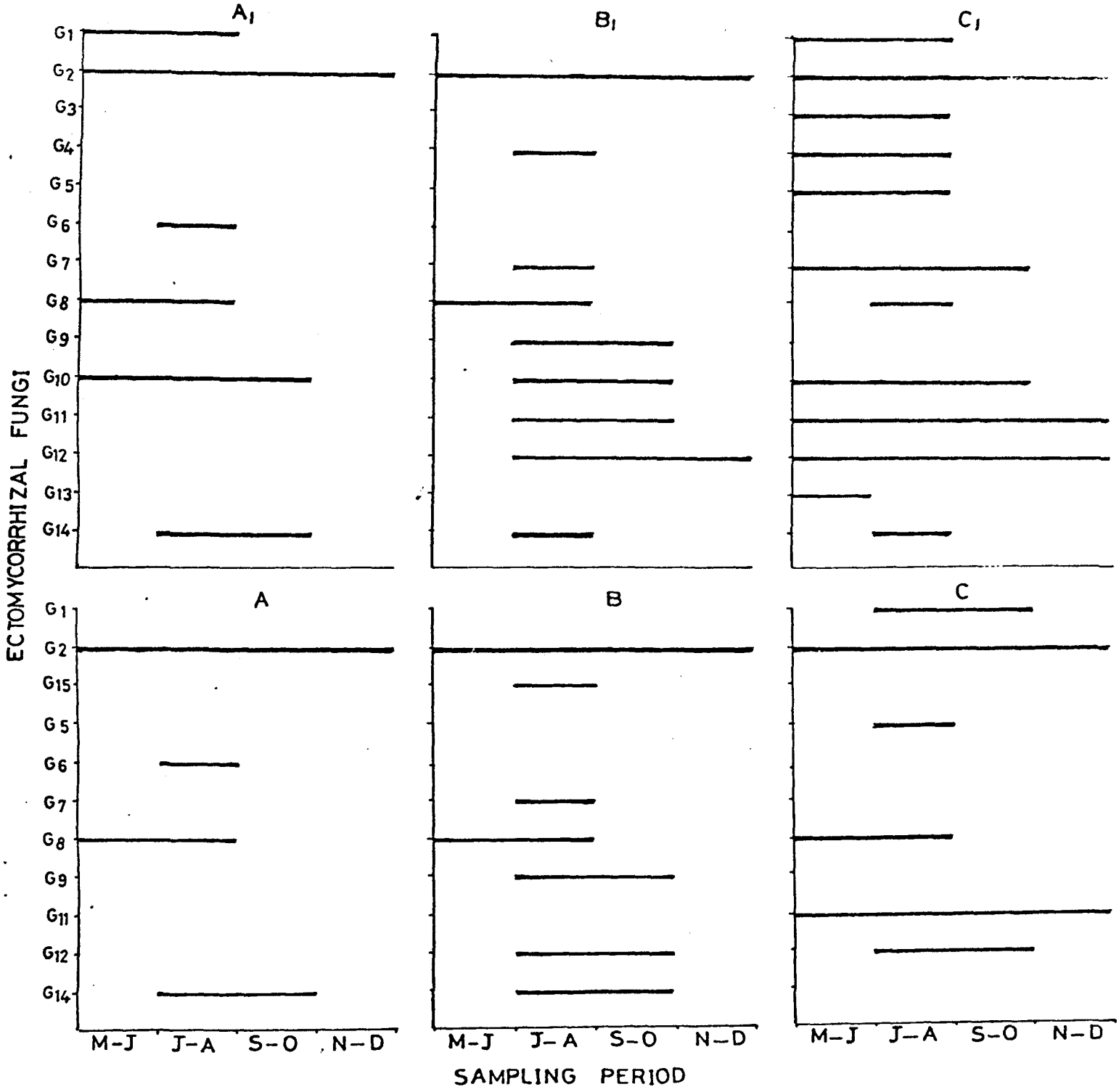


Fig. 1.3 : Occurrence of ectomycorrhizal fungi at different altitudes. A, B and C stand for higher, middle and lower altitudes, respectively, in 1987 and A_1 B_1 and C_1 stand for highest, middle and lowest altitude in 1988. G_1 - G_{14} for different fungi (see Appendix-1c).

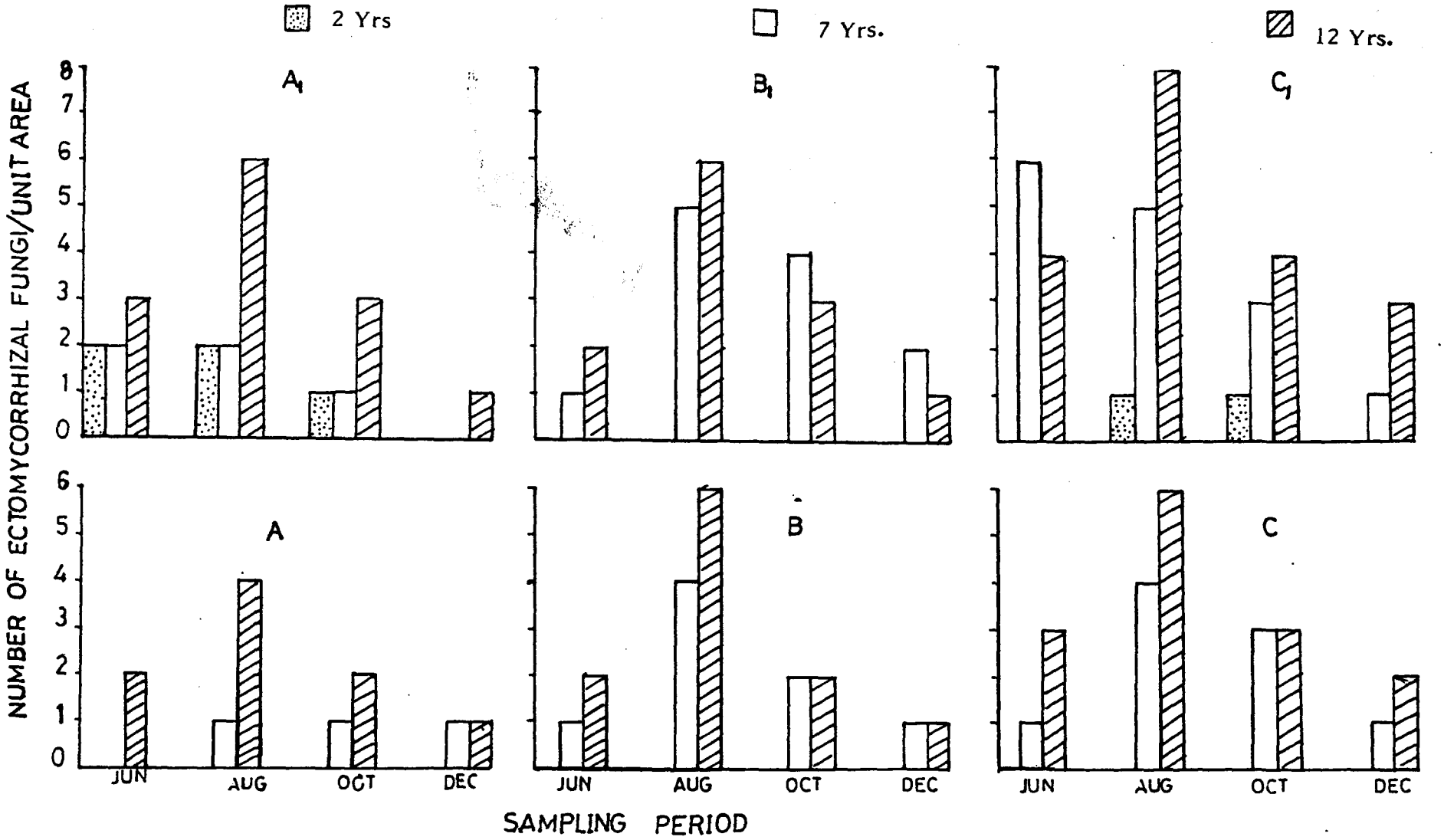


Fig. 1.4 : Population of ectomycorrhizal fungi at different altitudes A, B and C stand for upper, middle and lower altitudes in 1987 respectively and A_1, B_1 and C_1 stand for upper, middle and lower altitudes in 1988 respectively.

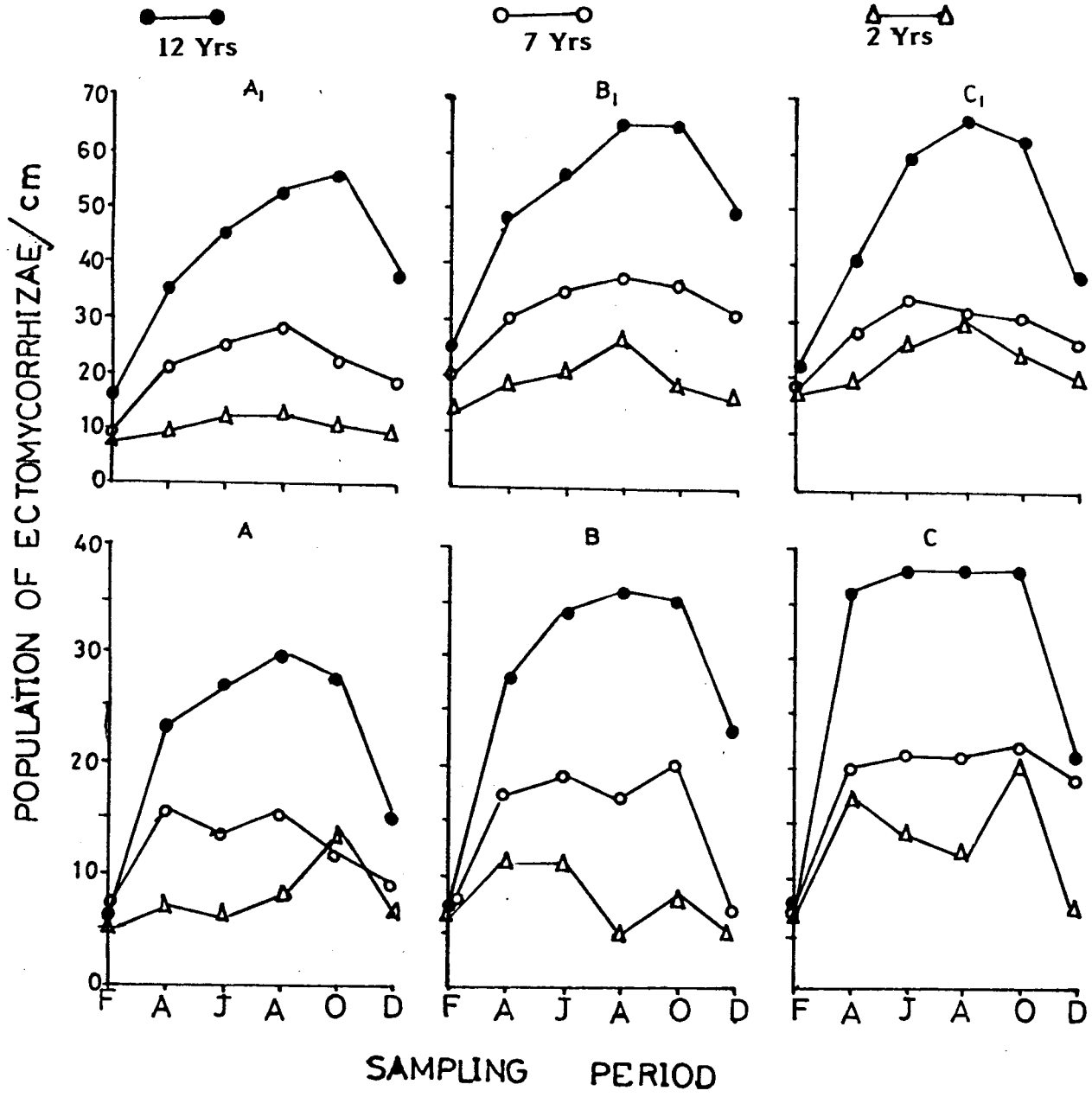


Fig. 1.5 : Population of ectomycorrhizae in different age groups of pine stand at different altitudes A - higher, B - middle and C - lower altitude in 1987 and A_1 - higher, B_1 - middle and C_1 - lower altitude in 1988.

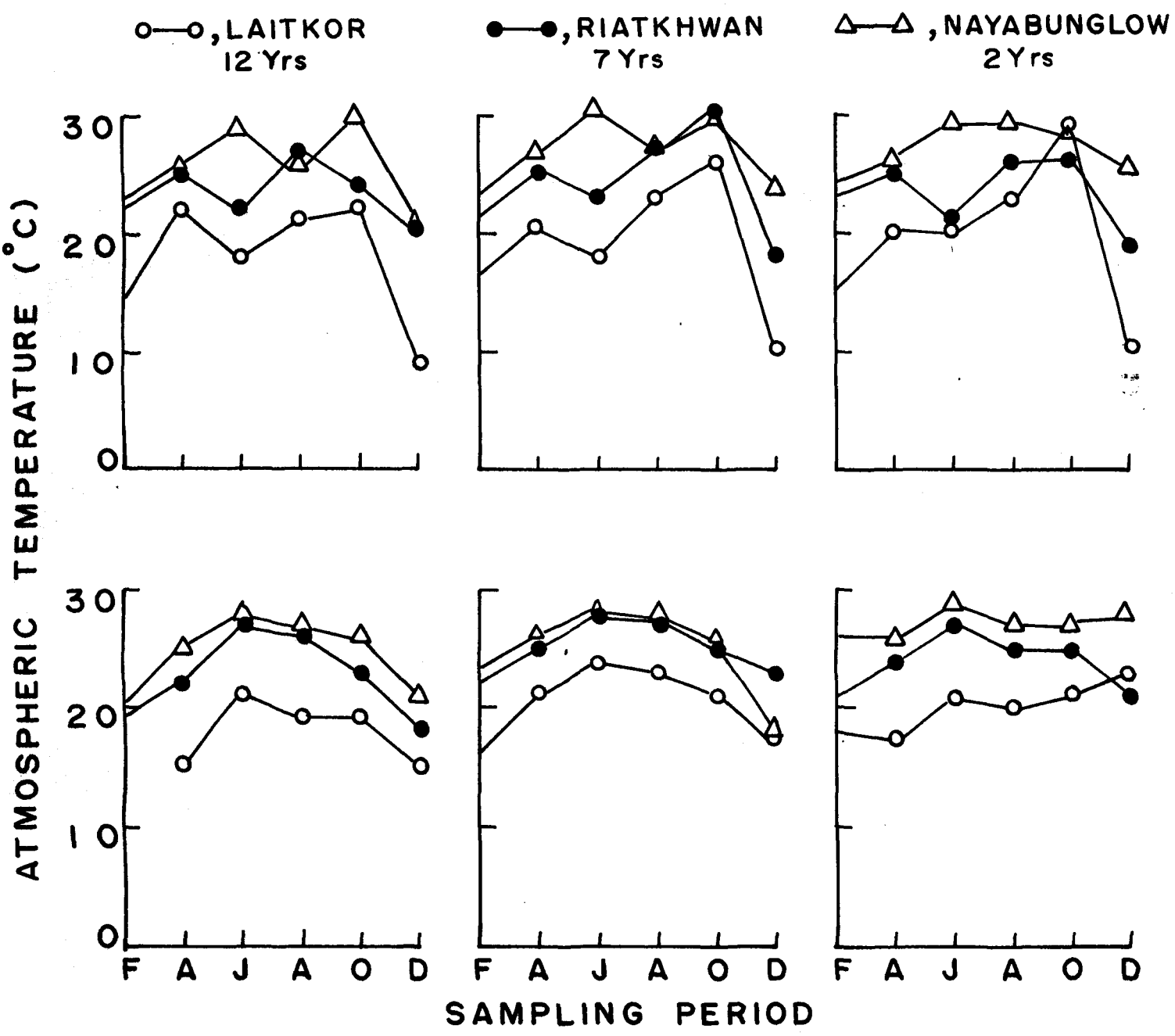


Fig. 1.6 : Atmospheric temperature (°C) of different study site. Lower and upper rows represent datas collected in 1987 and 1988 respectively. First, second and third columns (R to L) represent datas from 12, 7 and 2 years old pine stands.

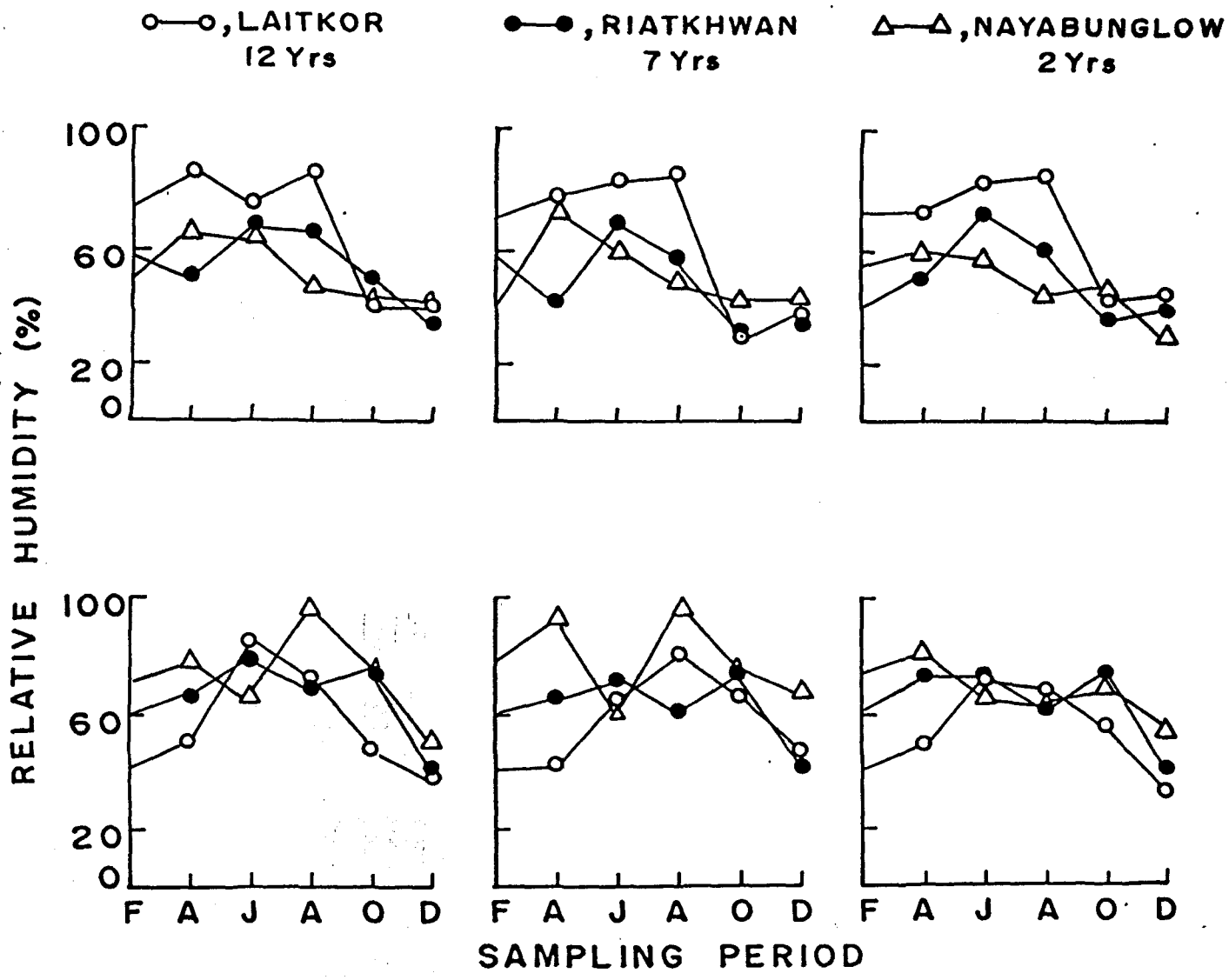


Fig. 1.7 : Relative humidity (%) of different study sites. Lower and upper rows represent datas collected in 1987 and 1988 respectively. First, second and third columns represent datas from 12, 7 and 2 years old pine stands.

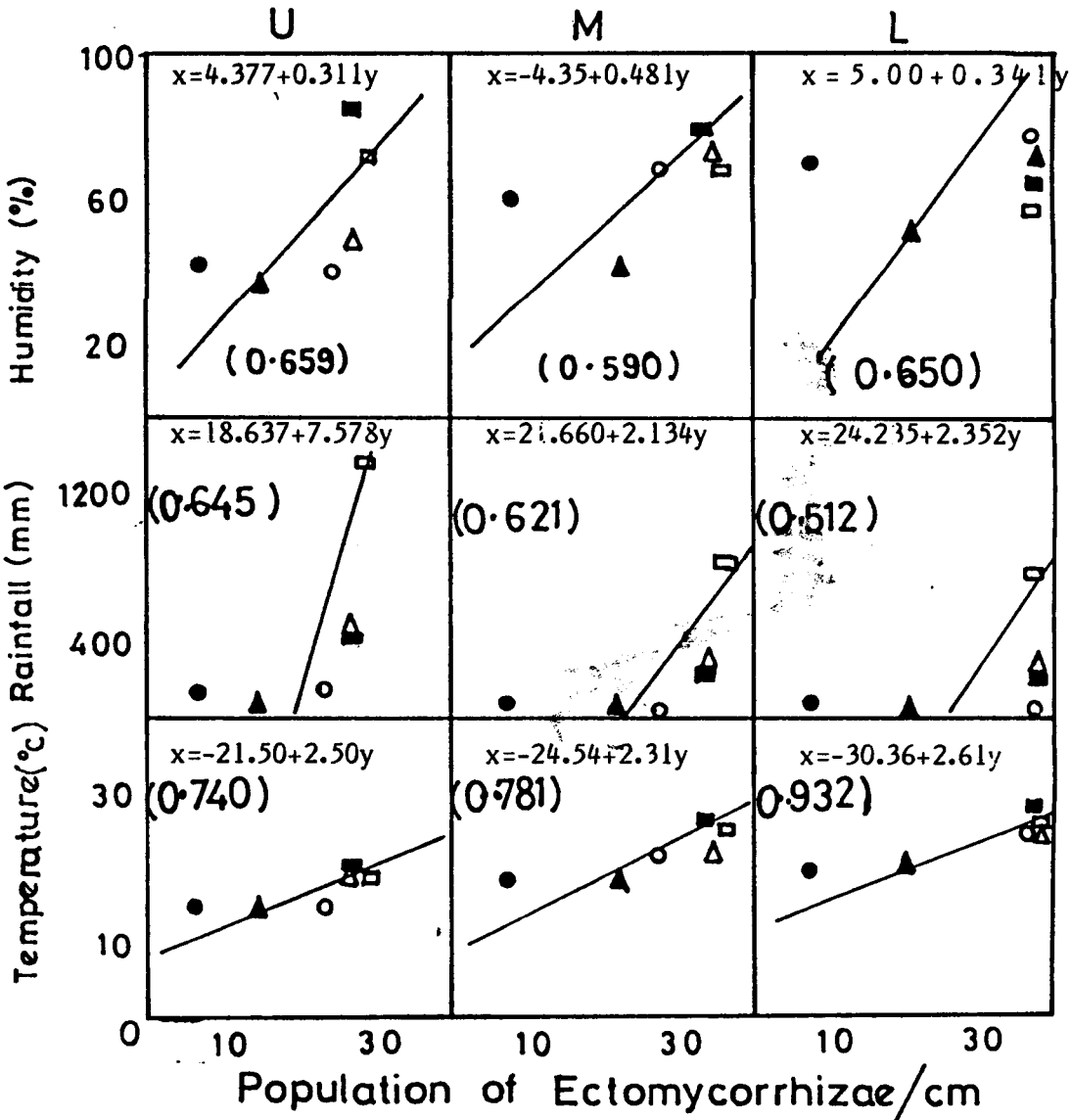


Fig. 1.8 : Correlation between population of ectomycorrhizae and humidity, rainfall, and temperature in 12 years old pine stand in 1987. Figures in parentheses represent the r value. (U=upper altitude, M=middle altitude and L=lower altitude).

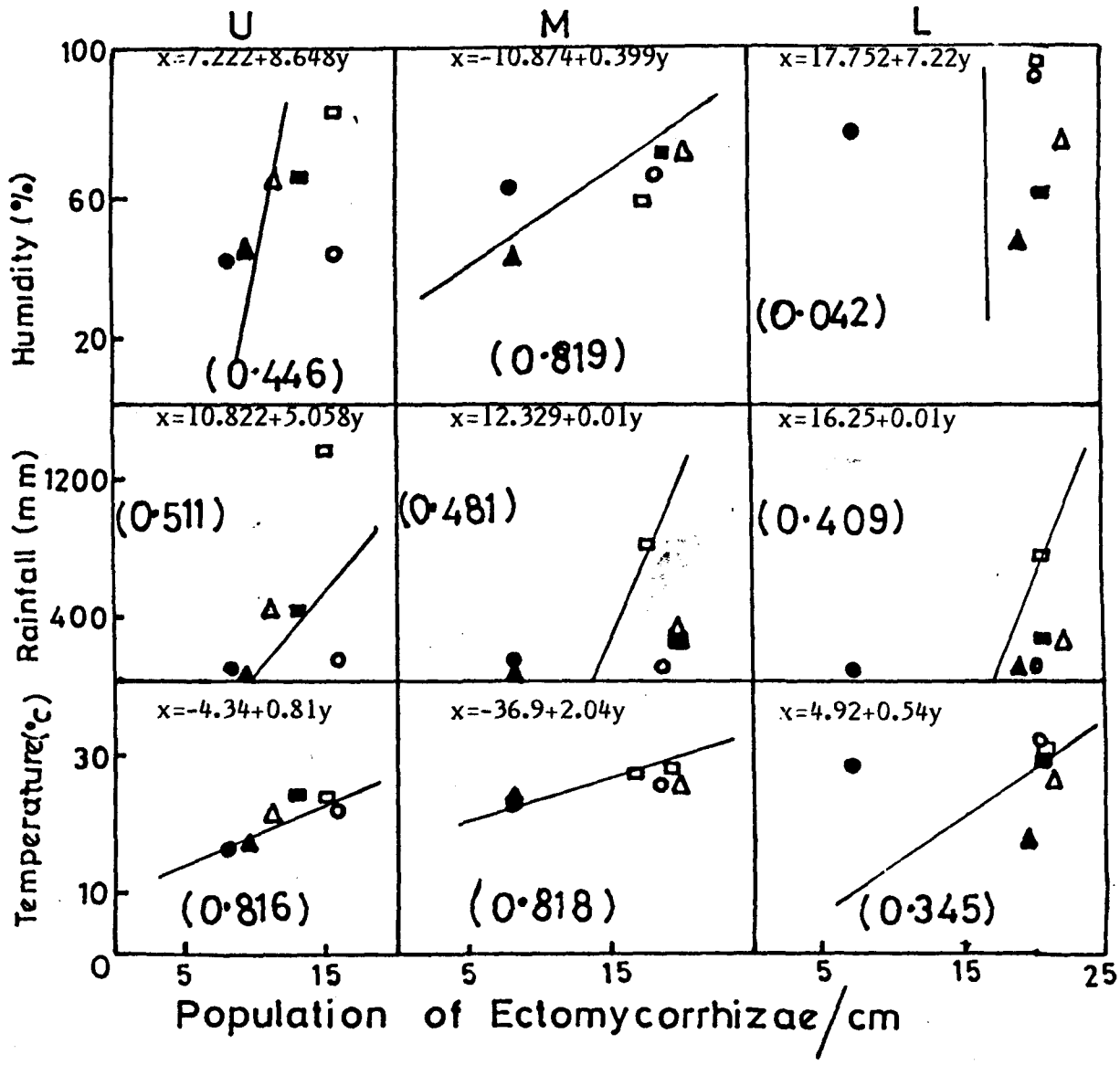


Fig. 1.9 : Correlation between ectomycorrhizae and humidity, rainfall and temperature in 7 years old pine stand in 1987. Figures in parentheses represent the r value. (U=upper altitude, M=middle altitude and L=lower altitude).

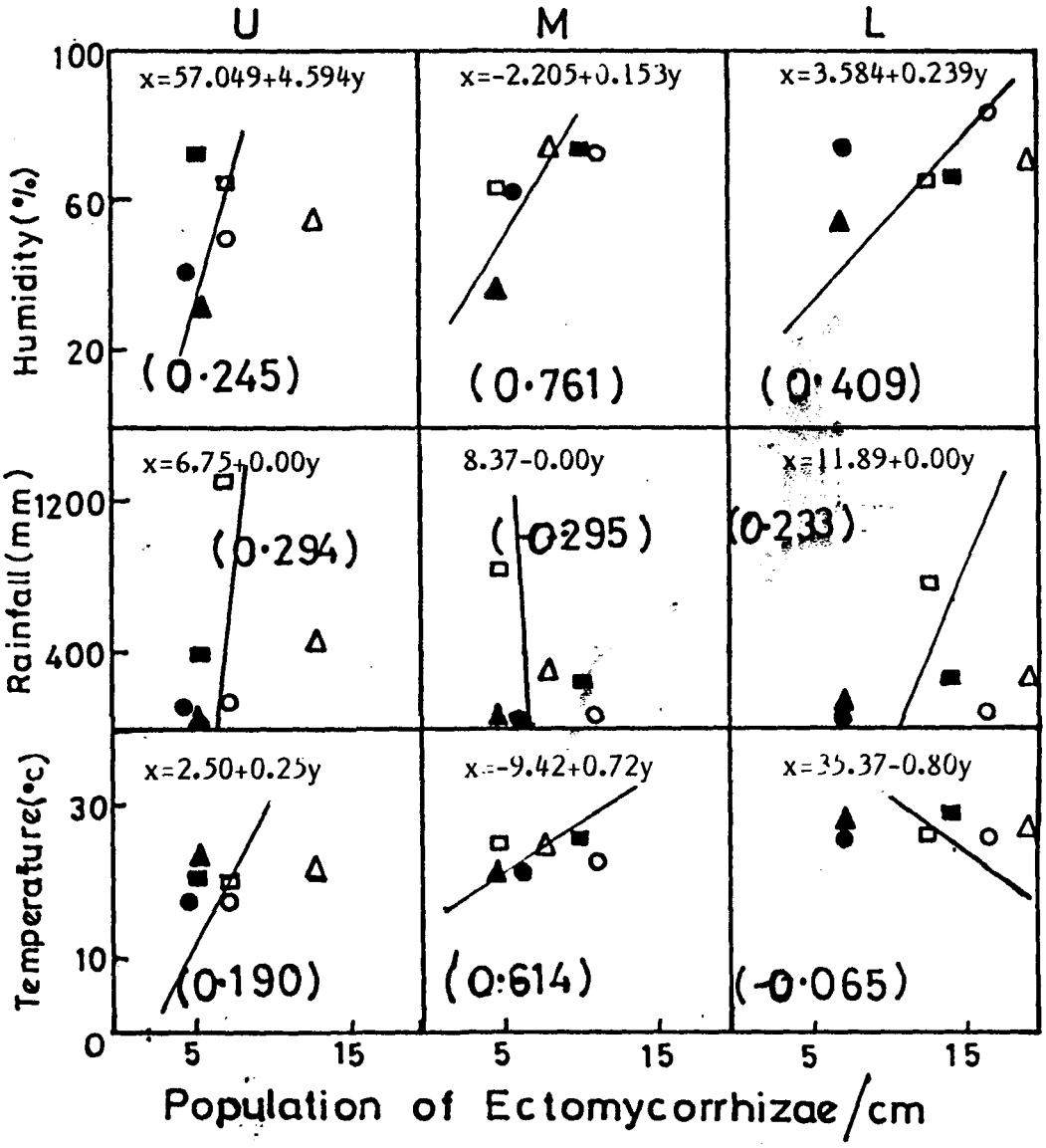


Fig. 1.10 : Correlation between ectomycorrhizae and humidity, rainfall and temperature in 2 years old pine stand in 1987. Figures in parentheses represent the r value. (U=upper altitude, M=middle altitude and L=lower altitude).

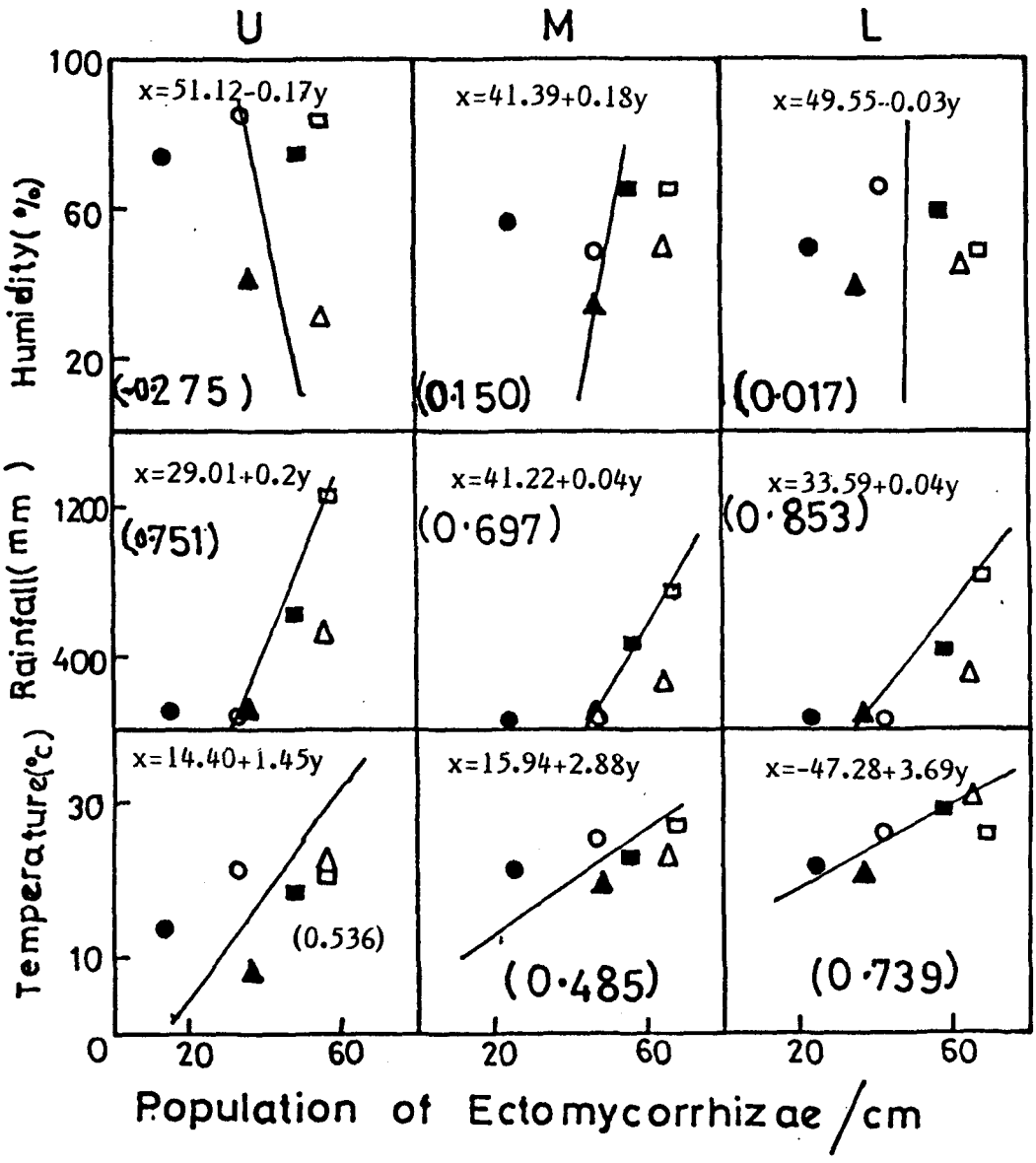


Fig. 1.11 : Correlation between ectomycorrhizae and humidity, rainfall and temperature in 12 years old pine stand in 1988. Figures in parentheses represent the r value. (U=upper altitude, M=middle altitude and L=lower altitude).

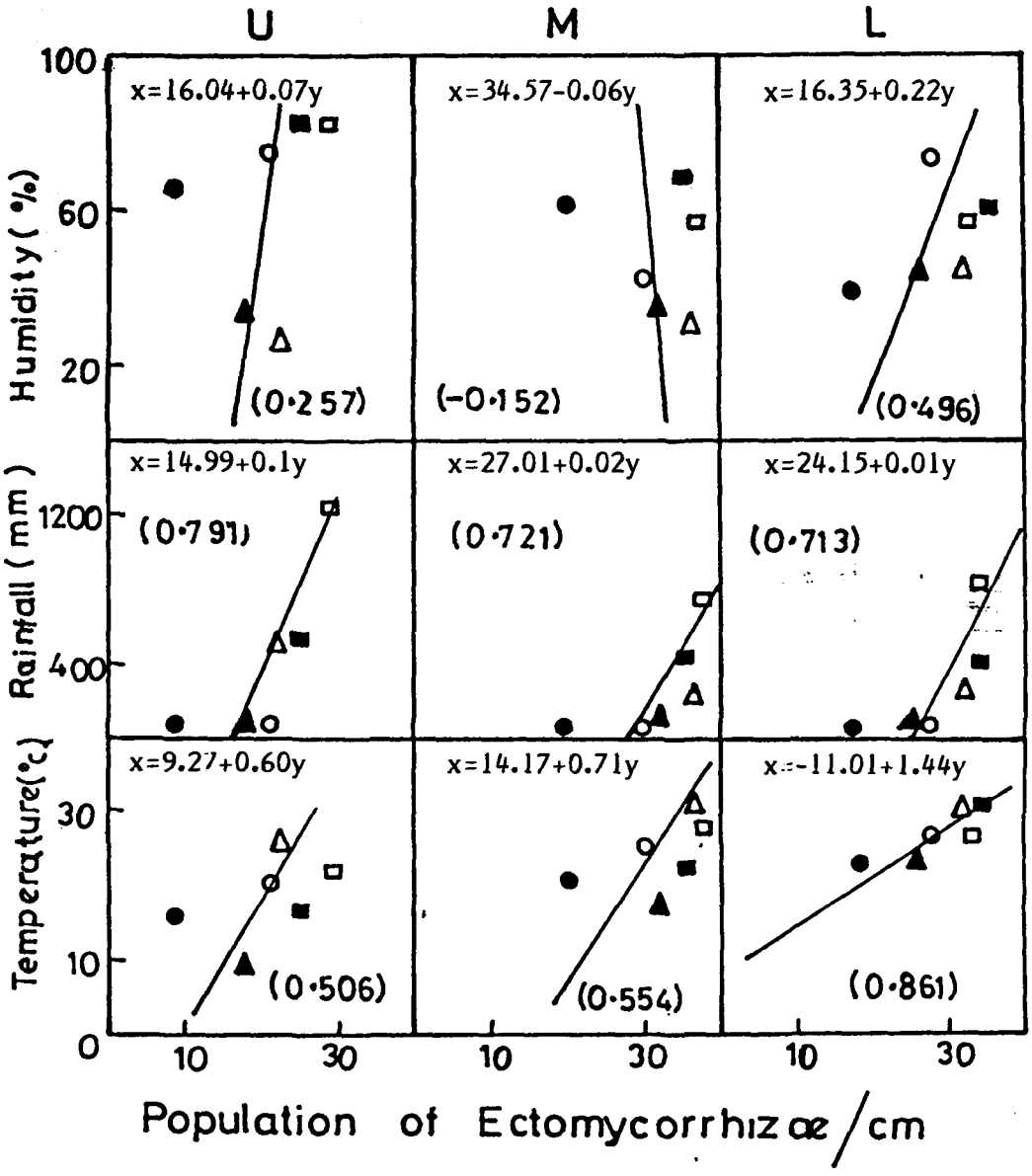


Fig. 1.12 : Correlation between ectomycorrhizae and humidity, rainfall and temperature in 7 years old pine stand in 1988. Figures in parentheses represent the r value. (U=upper altitude, M=middle altitude and L=lower altitude).

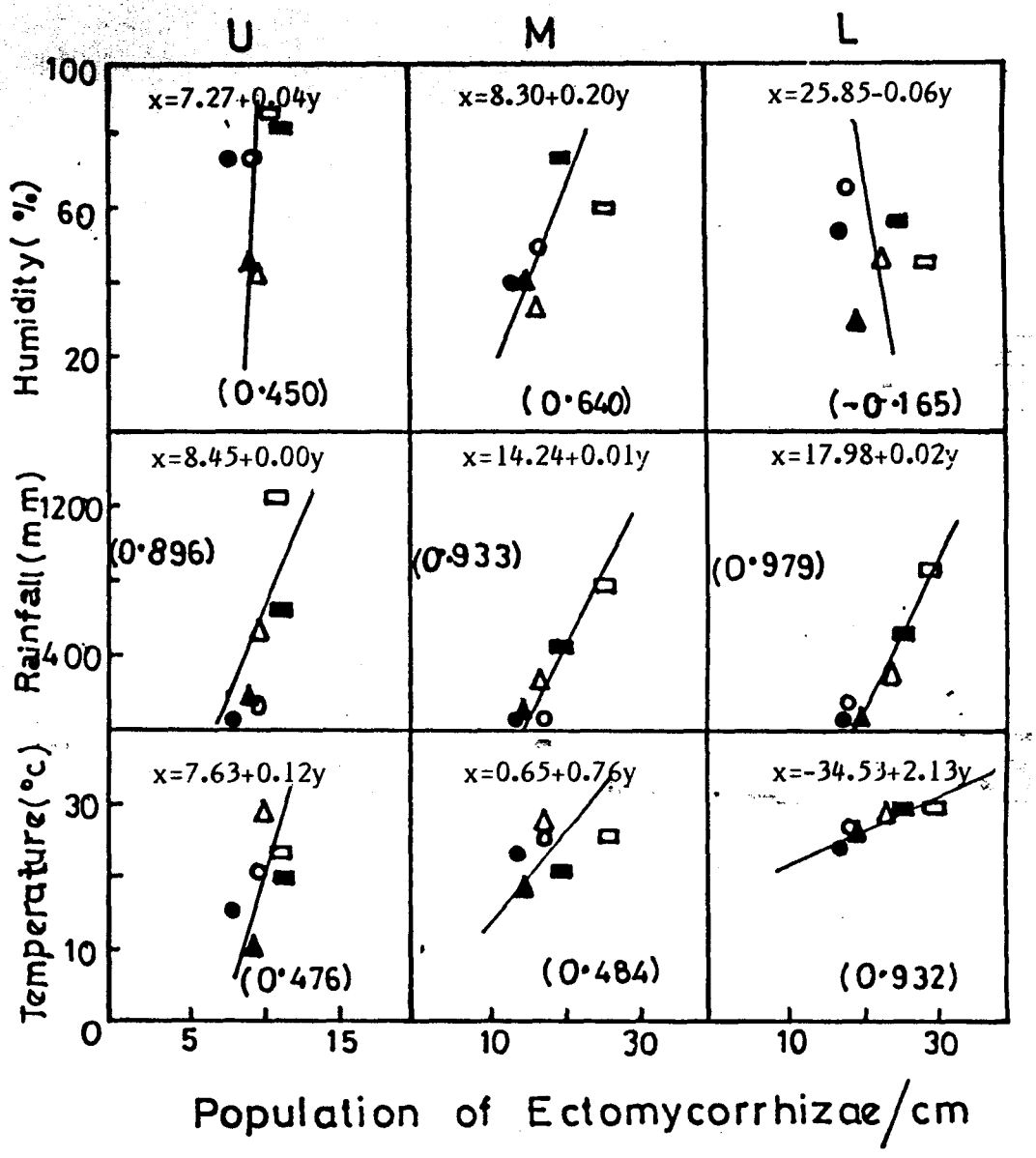


Fig. 1.13 : Correlation between ectomycorrhizae and humidity, rainfall and temperature in 2 years old pine stand in 1988. Figures in parentheses represent the r value. (U=upper altitude, M=middle altitude and L=lower altitude).

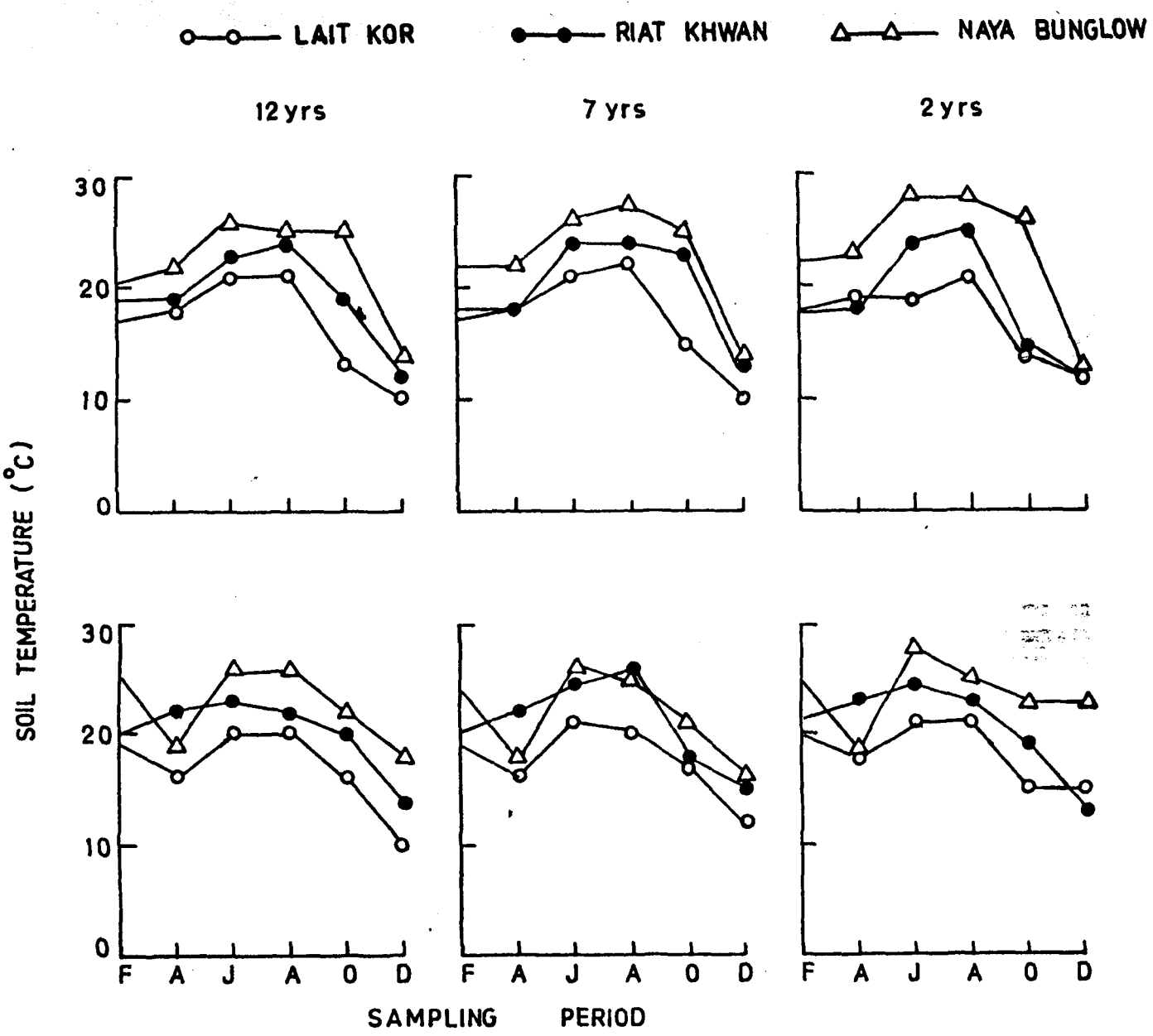


Fig.1.14 : Soil temperature (°C) of different study sites. Lower and upper rows represent datas collected in 1987 and 1988 respectively. First, second and third columns (R to L) represent datas from 12, 7 and 2 years old pine stands.

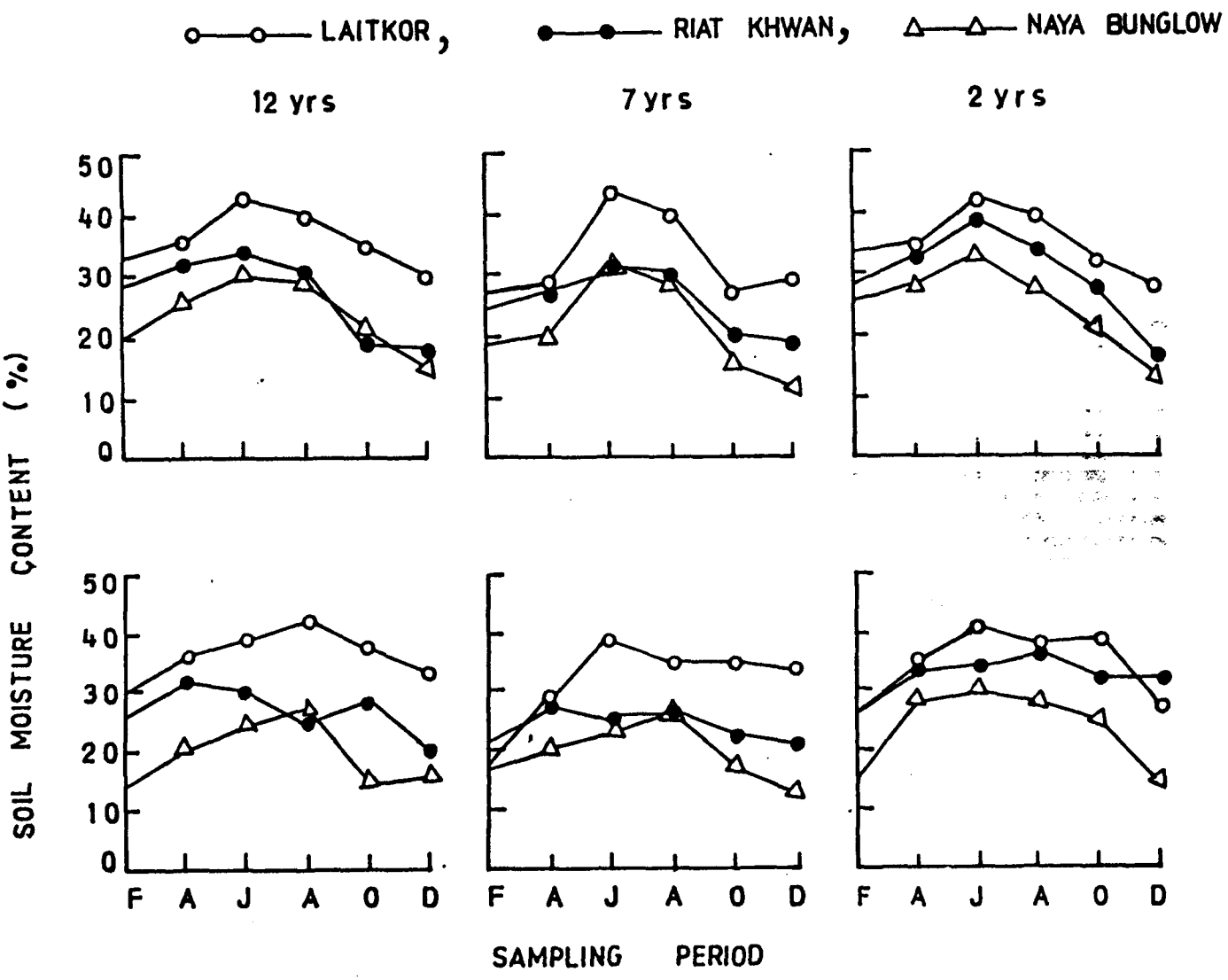


Fig. 1.15 : Moisture content (%) of the soils collected from different study sites. Lower and upper rows represent datas collected in 1987 and 1988 respectively. First, second and third columns (R to L) represent datas from 12, 7 and 2 years old pine stands.

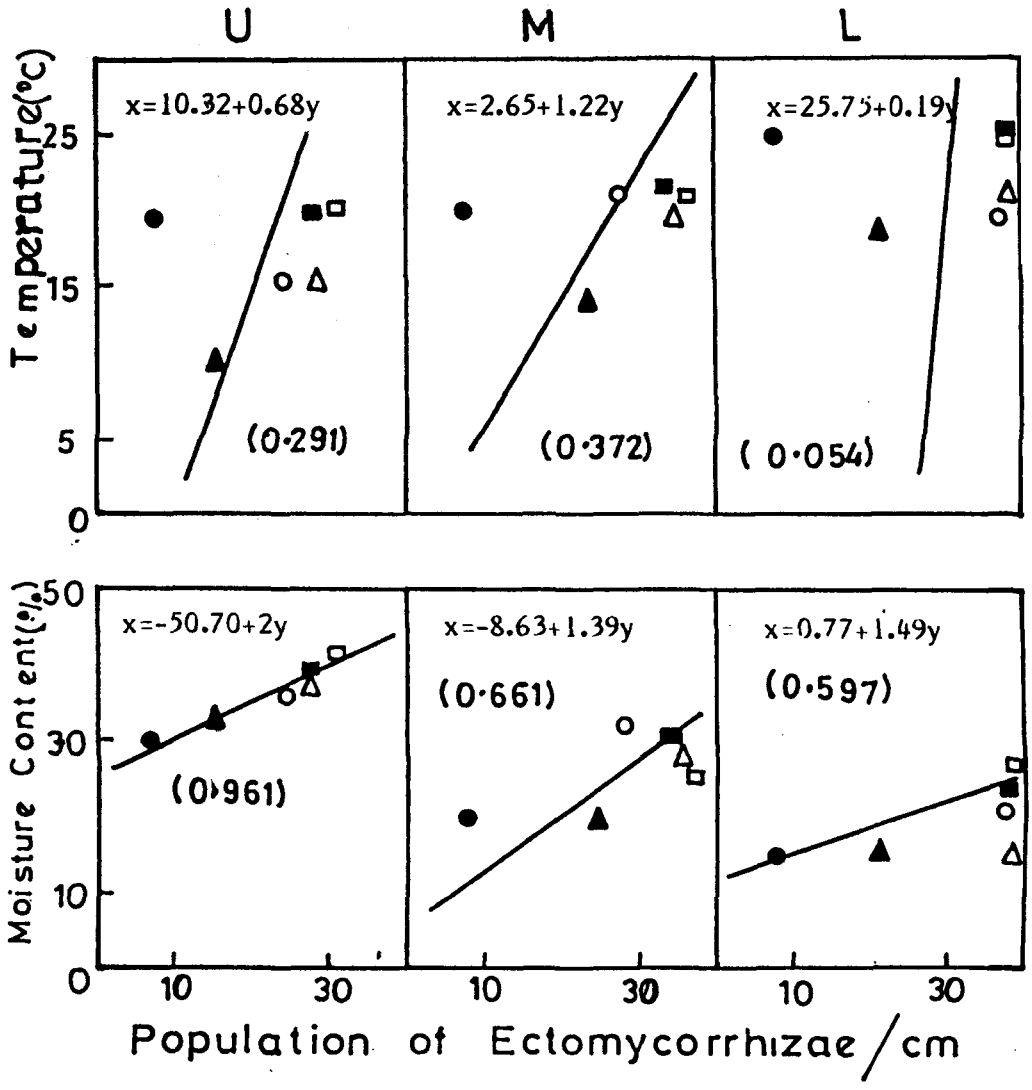


Fig. 1.16 : Correlation between ectomycorrhizae and soil moisture content and temperature in 12 years old pine stand in 1987. Figures in parentheses represent the r value. (U=upper altitude, M=middle altitude and L=lower altitude).

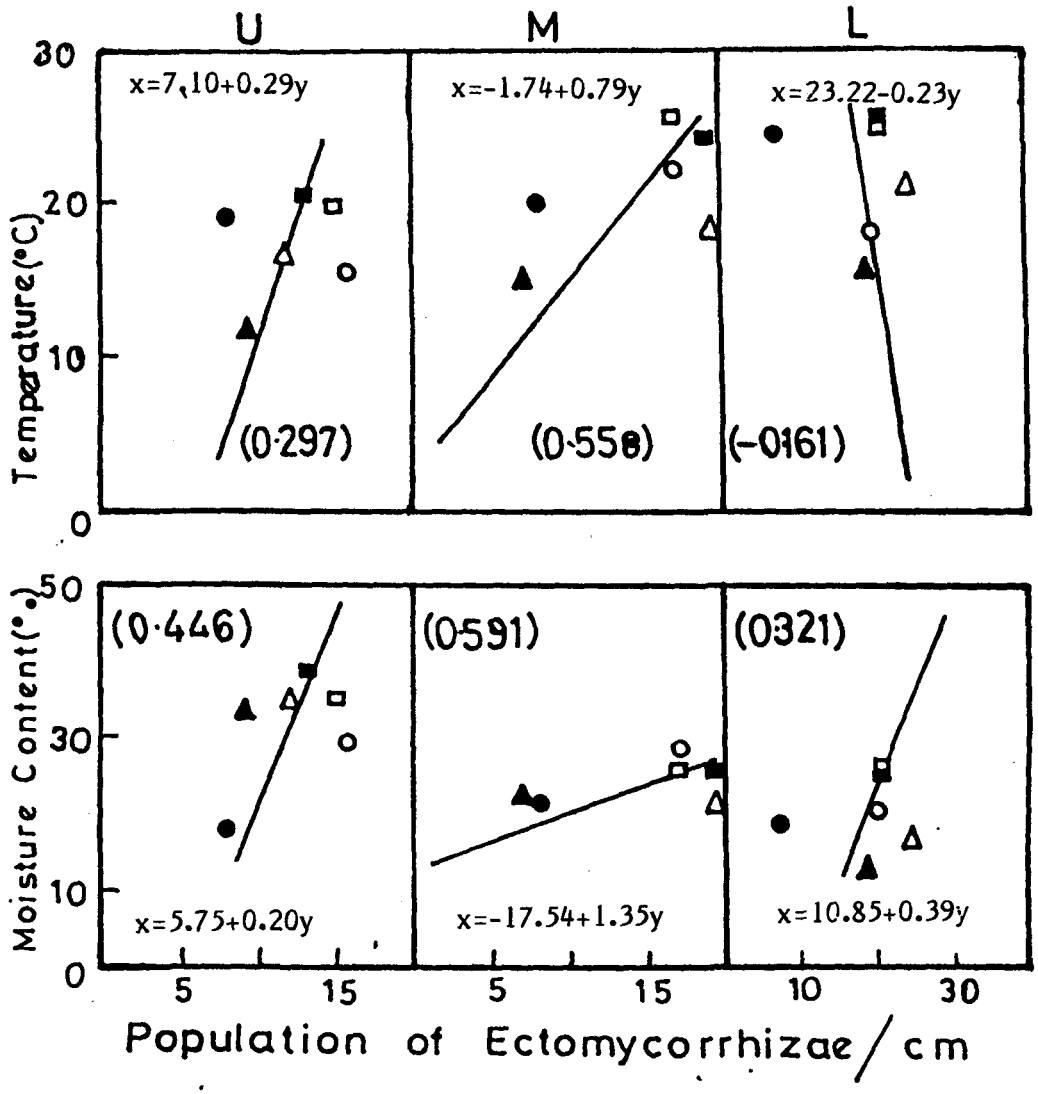


Fig. 1.17 : Correlation between ectomycorrhizae and soil moisture content and temperature in 7 years old pine stand in 1987. Figures in parentheses represent the r value. (U=upper altitude, M middle altitude and L lower altitude).

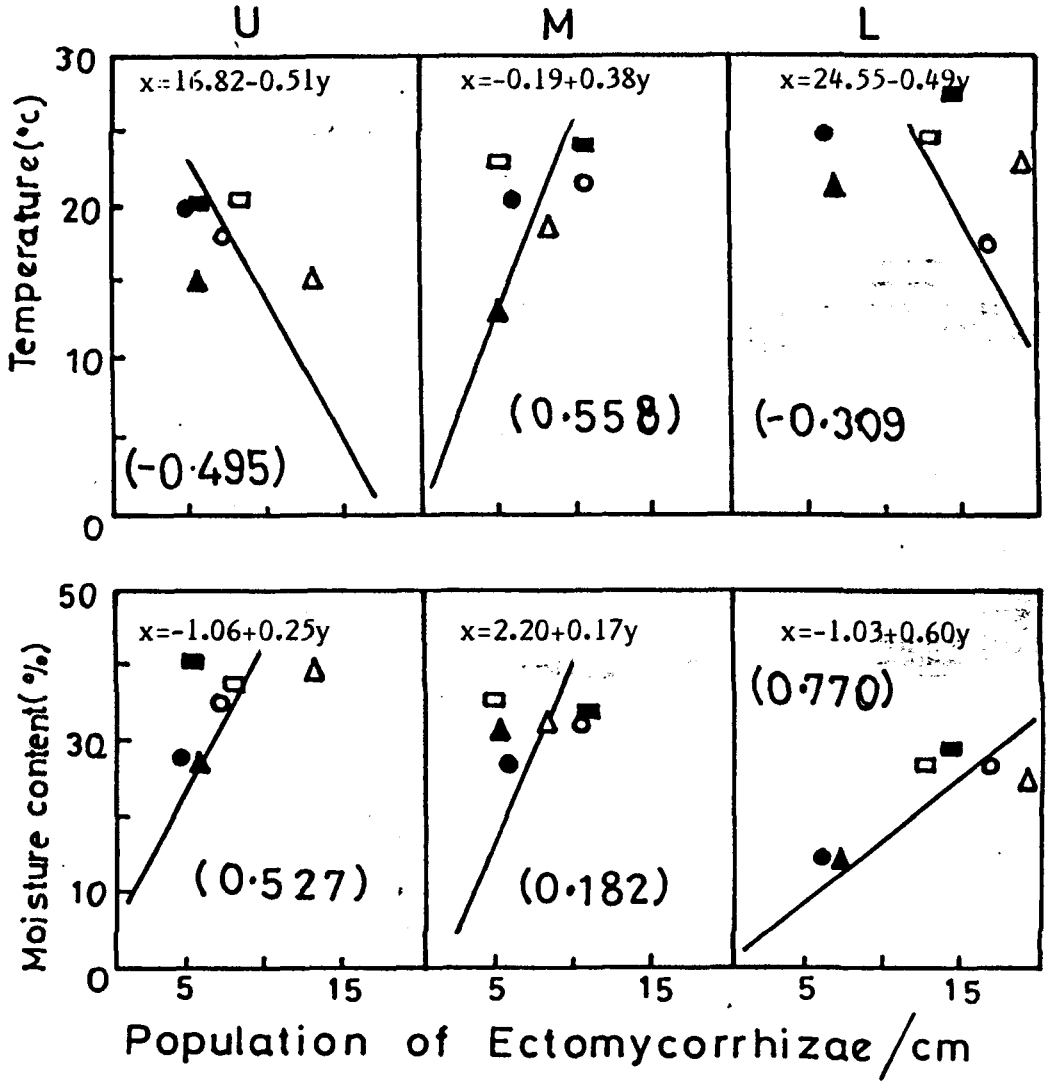


Fig. 1.18 : Correlation between ectomycorrhizae and soil moisture content and temperature in 2 years old pine stand in 1987. Figures in parentheses represent the r value. (U=upper altitude, M=middle altitude and L=lower altitude).

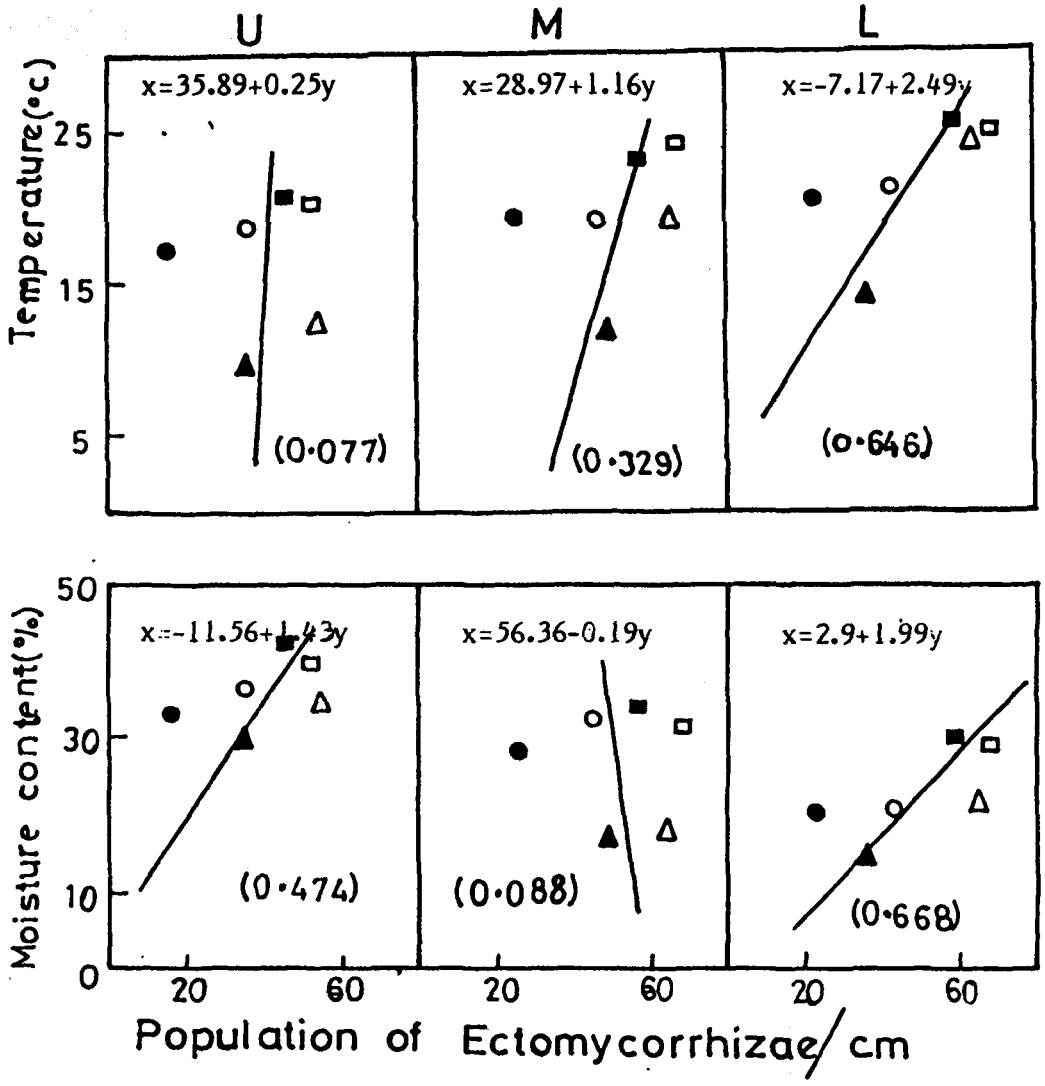


Fig. 1.19 : Correlation between ectomycorrhizae and soil moisture content and temperature in 12 years old pine stand in 1988. Figures in parentheses represent the r value. (U=upper altitude, M=middle altitude and L=lower altitude).

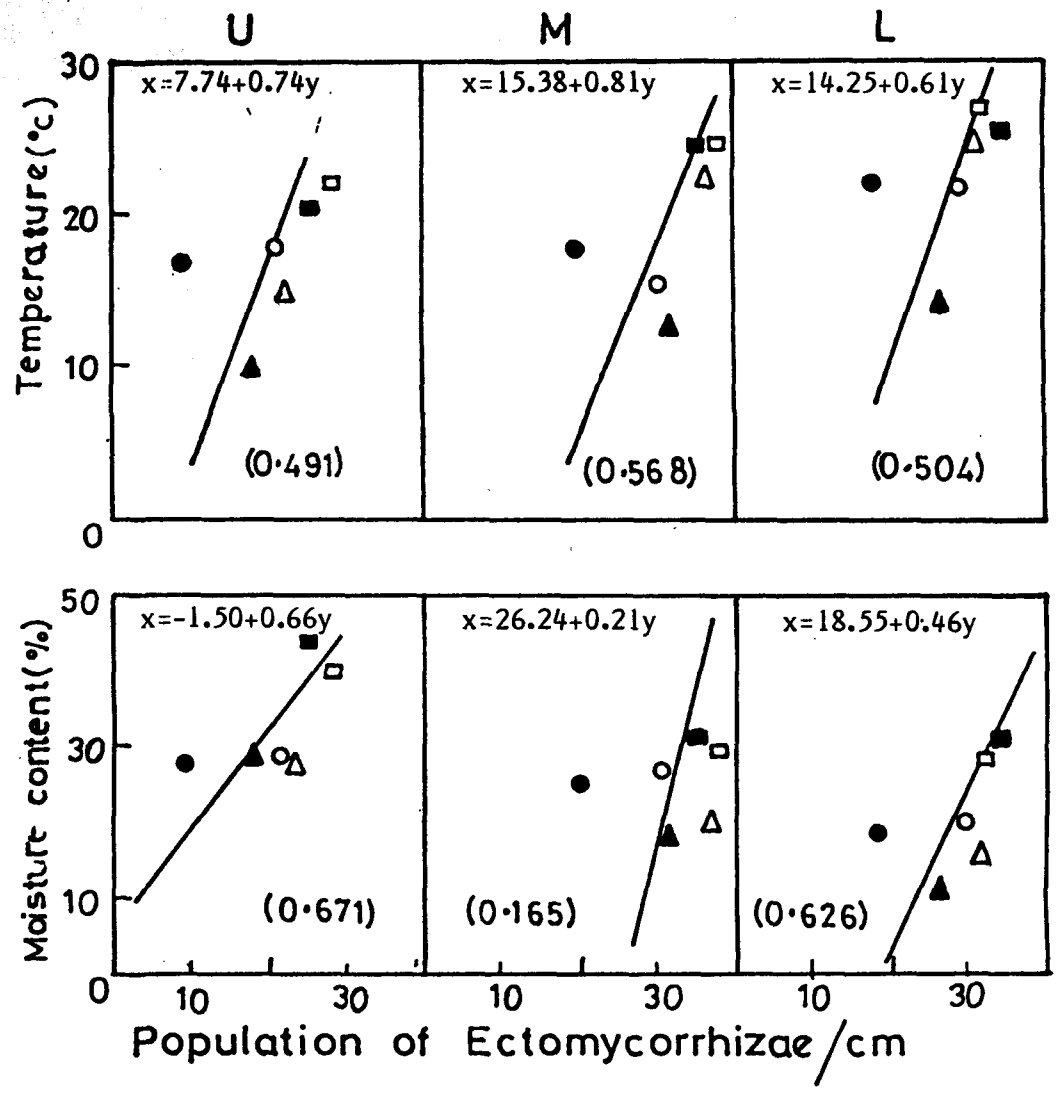


Fig. 1.20 : Correlation between ectomycorrhizae and soil moisture content and temperature in 7 years old pine stand in 1988. Figures in parentheses represent the r value. (U=upper altitude, M=middle altitude and L=lower altitude).

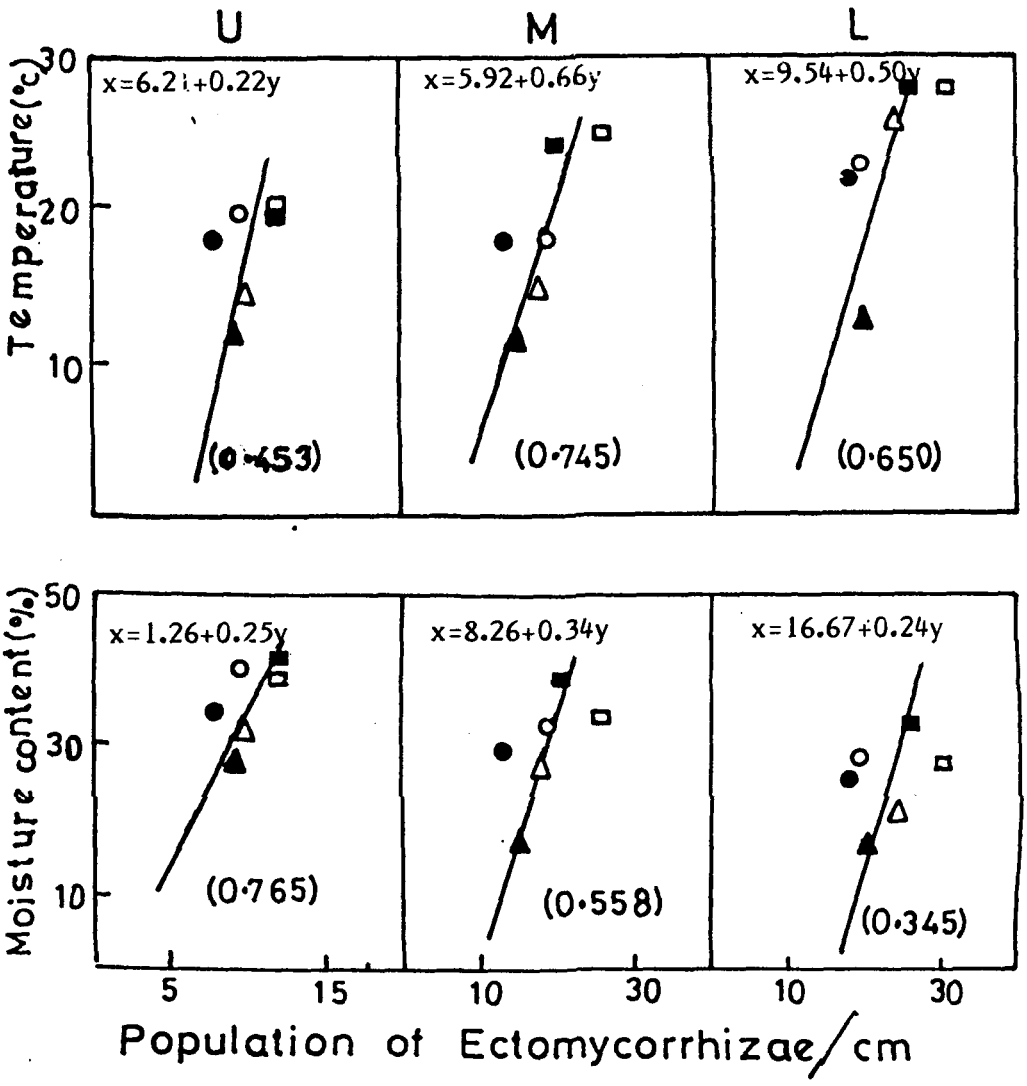


Fig. 1.21 : Correlation between ectomycorrhizae and soil moisture content and temperature in 2 years old pine stand in 1988. Figures in parentheses represent the r value. (U=upper altitude, M=middle altitude and L=lower altitude).

CHAPTER - II

ISOLATION TECHNIQUE, MAINTENANCE AND MASS CULTURE OF ECTOMYCORRHIZAL FUNGI

Introduction

A number of fungi forms ectomycorrhizal association with pines in natural conditions. They help the seedlings in making nutrients available from the unavailable source in the poor soils. For better understanding of their aut-ecology and physiology, it is necessary to obtain them in pure culture. After the classical work of Frank (1885), some of these fungi have been isolated by few workers (Chu-chou, 1979; Chu-chou and Grace, 1980, 1981, 1988; Ng et al., 1982, and Malajczuk et al., 1982), but some problems in their isolation still exist. In India also, some work on the isolation of mycorrhizal fungi from mycorrhizae has been initiated (Bakshi, 1974; Sharma, 1981; Sharma and Mishra, 1988). However, a detailed study on isolation of the ectomycorrhizal fungi and their growth under different climatic conditions is lacking.

Before confirming the mycorrhizal relationship of fungal isolate obtained either from sporocarps or mycorrhizal roots, it is necessary to resynthesize the mycorrhizae and reisolate them from the synthesized mycorrhizal roots.

Ectomycorrhizal fungi are slow growers on the synthetic media (Chu-chou and Grace, 1981). While carrying the work on isolation of symbionts, fast growing forms may either suppress or inhibit their growth. Few attempts to avoid the microbial contamination have been made by the use of surfactant and antibiotics

(Chu-chou, 1979; Sharma, 1981; Chu-chou and Grace, 1988). However, very less success has been achieved in this regard.

Some factors like nutrition (Chu-chou, 1979; Sharma, 1981; Ng et al., 1982), temperature (France and Reid, 1979 and Cline et al., 1987) and source of isolation (sporocarps and mycorrhizal roots) (Lamb and Richards, 1970 and Chu-chou and Grace, 1988) have been studied to know about the suitable conditions for the optimum growth of mycobiont.

A suitable technique is, however, required for the maintenance of ectomycorrhizal fungi to use them for further experimental works. These fungi can either be maintained at a low temperature or on a rich nutrient medium (Sharma, 1981; Ng et al., 1982). However, subculturing and loss of viability of these fungi may cause some problems (Ng et al., 1982).

Fast multiplication and mass production of ectomycorrhizal fungi on a large/mass scale is necessary to inoculate the seedlings in nursery, afforestation and plantation programmes. Introduction of pure mycelial culture of mycobionts has been used as the most common method in afforestation programmes (Bowen, 1965; Mikola, 1973; Trappe, 1977; Marx et al., 1980). However, the method has limited application and is not suitable for larger plantation programmes (Raman, 1988; Sharma and Mishra, 1988). Works on developing some suitable methods to produce mass inoculum are under progress (Raman, 1988).

Informations on the isolation, maintenance and mass culture of mycorrhizal fungi from Pinus kesiya Royle ex. Gordon are very meagre (Sharma, 1981). It was, therefore, planned to develop the technique to isolate, maintain and produce mass culture of dominant ectomycorrhizal fungi, which can be exploited to assess their efficiency in improving nutrient uptake and growth of the seedlings of pine.

Material and Methods

(i) Isolation of ectomycorrhizal fungi from roots :

Different pine stands were selected for the isolation of ectomycorrhizal fungi (mentioned in Chapter I). A monolith (15 cm x 15 cm x 15 cm) was sampled keeping a distance of 1.5 metres, 1 metre and 0.5 metre from the pine tree trunks in 12, 7 and 2 years old pine stands respectively in five replicates. The soil samples were brought to the laboratory on the same day. Thereafter, they were washed under tap water in netted trough. The mycorrhizal roots were sorted out and collected in cleaned petridish. The young white ectomycorrhizal roots were further sorted out and transferred to sterilized specimen tubes. The roots were thoroughly washed again under tap water. Washed root segments were examined under binocular sterio microscope for fungal association. Young white light coloured terminal portions of the mycorrhizal roots were picked up. Fifty such pieces (5 mm) were transferred to another sterilized specimen tubes containing few drops of liquid detergent (Teepol, BDH) in sterilized water. The specimen tubes were shaken vigorously for 5 minutes. Thereafter, root segments were transferred to a 250 ml conical flask containing 150 ml distilled water and shaken vigorously to wash the root segments. Five to six washings were given before surface sterilization with 1% solution of sodium hypochlorite for 10 minutes. The root segments were then given five to six washings with sterilized water and transferred to a sterilized petridish under aseptic condition. The root segments were teased with the help of sterilized needles.

Different nutrient agar media (mentioned in Appendix II) were prepared and poured into sterilized petridishes and test tubes under aseptic conditions. Slants were made in the culture tubes. Five teased root segments were inoculated

in a petridish with the help of sterilized inoculation needle in 25 replicates on each medium. One teased root segment was inoculated into a slant in culture tube in 50 replicates separately for each medium. Inoculated petridishes and tubes were incubated at 23°C (± 2°C) for 25 days in dark condition in a BOD incubator. Afterwards, the culture plates and tubes were examined for the growth of ectomycorrhizal fungi. Contaminated plates and tubes were discarded. The cultures of fungi thus obtained were then transferred to freshly prepared slants of modified Melin-Norkrans agar medium and incubated for 10 days at 23°C (± 2°C) (Marx, 1969).

(ii) Isolation of mycorrhizal fungi from sporocarps :

Developing fruit bodies of basidiomycetes growing in a pine forest were traced for their hyphal connection with the roots of pine and collected in a sterilized polythene bag. Fruiting bodies were brought to the laboratory on the same day and identified with the help of Singer's book (1962). Their abexial surfaces were sterilized with 90% alcohol for one or two minutes. After that fruiting bodies were cut into two halves with the help of sterilized scalpel. Small pieces of tissue were separated from the inner side of pieced cap of fruit body and inoculated onto modified Melin-Norkrans agar medium in 20 replicates. The petriplates were incubated at 23° (± 2°C) for 25 days in the BOD incubator under dark condition. The whole isolation process was carried out under aseptic condition.

The plates were checked regularly and the contaminated ones were discarded. The cultures were examined under microscope for clamp connection and were sorted out.

(iii) Maintenance of the ectomycorrhizal fungi :

The ectomycorrhizal fungi were purified by hyphal cut method and were

multiplied on modified Melin-Norkrans nutrient agar medium (MMN). The pure cultures of fungi were then transferred onto the slants of MMN medium in culture tubes and allowed to grow for five to six days at 23°C (± 2°C) in the BOD incubator. Twenty-five culture tubes in each case were taken out after six days of incubation and kept at 5°C in a refrigerator. At a regular interval of 60 days, the tubes were replaced by freshly prepared MMN medium.

(iv) Synthesis of ectomycorrhizae by isolated cultures :

To confirm the isolated fungi as mycorrhizal, their symbiotic association was resynthesized under laboratory condition.

The pine seeds were soaked in a sterilized water for 24 hours and surface sterilized in 5% sodium hypochlorite for 5 minutes. The seeds were washed with sterilized water 5 times and transferred to sterilized moist chamber. These moist chambers were then kept at 25°C temperature in dark condition for seed germination. After 20 days, seedlings of 2 cm long radicle were transferred to the culture tubes (19.5 x 2.2 cm) containing 30 ml (at the time of pouring) agar thiamine medium (please see Appendix II). Fungi isolated earlier from roots/sporocarps were grown on MMN medium at 23° (± 2°C) for 30 days.

After 10 days of seedling transplantation in culture tubes, the selected isolates of fungi were inoculated (5 mm diameter block) near the roots of pine seedlings in ten replicates and incubated at 20°C (± 2°C) under light (2500 lux) of 12 hours photoperiod. The relative humidity was maintained (70-90%) in the growth chamber.

The seedlings were observed at monthly interval for the development of mycorrhizal association. Reisolation of ectomycorrhizal fungi was done from the newly synthesized mycorrhizal roots.

(v) Mass culture of the ectomycorrhizal fungi :

The ectomycorrhizal fungi maintained in pure cultures at 5°C in the refrigerator were taken out for multiplication. Based on the observations (please see the results) for mass culture of ectomycorrhizal fungi, the MMN nutrient medium was selected. The modified Melin-Norkrans medium was prepared, sterilized and poured in the petridishes. The plates were inoculated with different species of ectomycorrhizal fungi for inoculum preparation. These plates were incubated at 23°C (± 2°C) in the BOD incubator for 25 days. The MMN liquid medium was prepared on large scale (pH 5.5 with the help of .1N H_2SO_4 and .1N NaOH). 250 ml liquid medium (MMN) was poured into conical flask (500 ml) in 25 replicates for each fungal species and sterilized at 15 lb/inch pressure for 30 minutes and allowed to cool. Five millimeter block was cut with the help of sterilized cork borer from the grown plates of ectomycorrhizal fungi and transferred to conical flask (500 ml) under aseptic conditions in 30 replicates. These conical flasks were incubated at 25°C (± 2°C) under dark condition. Culture flasks were shaken at a regular interval. After 30 days, fungal cultures covered the open surface of the liquid medium. Fungal cultures were filtered, washed with the help of sterilized water and homogenized with sterilized water (1:2 W/v) in Metrex tissue homogenizer.

Results

Modified MelinNorkrans and Hagem's nutrient media were found better for the growth of ectomycorrhizal fungi than other media. MMN's medium was comparatively better than Hagem's ones. Mass culture of the symbiont was, therefore, performed only on MMN medium either supplemented with peat moss or with vermiculite.

Five ectomycorrhizal fungi were isolated from different pine woodlands

and tested for their synthesis of mycorrhizal association (Table 2.2). Four ectomycorrhizal fungi were isolated from the sporocarps and one from the mycorrhizal root of pine (Table 2.1). Efforts were made to get fruiting bodies of Cenoccocum sp. for assigning their perfect stage but no success could be achieved. It was, therefore, characterized and indentified based on morphological characters of vegetative mycelium. Some morphological characters of sporocarps and the radial growth was used for the identification of fungi using the keys of Singer (1962, 1975), Chinery (1983) and Reid (1969, 1972). We could not isolate all the mycorrhizal fungi observed under natural pine stand (Chapter I of the thesis). Only those species which grew on nutrient media are described here.

Pisolithus tinctorjus

Sporophore upto 11 cm high, subglobose; stipe long and smooth with dark brown colour; base burried into the ground with a brown mycelial threads; upper portion of the gleba formed by crowded pea sized peridioles with brown colour; odour pleasant mushroomy; spores brown, globose and 7-10 microns.

Season - Later part of rainy season.

Fungal colony brown, buging with profuse aerial hyphae; mycelia loosely interwoven; hyphal size 2.9 $\pm$ 0.25 μm; mycorrhiza brownish and dichotomously branched.

Cenococcum sp.

Fungal colony black, round with regular margin; rough in texture; aerial hyphae absent; open surface of the colony wet; growth of the fungus extremely slow on the nutrient media; black colour of the fungal mycelia make the substrate black; mycelia compactly interwoven; form the sclerotia under dried condition; hyphal size 8.2 $\pm$ 0.31 μm ; mycorrhiza black and digitate with rough texture.

Scleroderma aurantium

Carpophore diameter 8-10 cm, subglobose; peridium very thick, split into polygonal scales, coarse in texture; gleba blackish; odour strong; spores brown, globose, spiny, 4-9 microns.

Season - Rainy season.

Fungal colony grey, rough in texture, aerial hyphae absent; mycelia closely interwoven; warty appearance with irregular margin; hyphal size 7.6 $\pm$ 0.27 µm; mycorrhiza dichotomously branched and white with smooth texture.

Boletus edulis

Sporophores 5-10 cm high; cap 5-7 cm, convex, smooth, viscous in damp weather, yellowish in young stage turning light brown towards maturity; pore small, circular; stipe smooth; flesh white and soft; odour pleasant; spores olive brown, fusiform, 4-6 microns.

Season - Rainy season.

Fungal colony light brown; aerial hyphae present; mycelium closely interwoven; hyphal size 9.3 $\pm$ 0.41 µm ; mycorrhiza coralloid and creamy with smooth texture.

Suillus sp.

Sporophore 4-10 cm high; cap 5-6 cm, convex, presenting conical appearance; margin regular with partial velar remains; cuticle viscous in damp weather, greyish brown; pore tiny, polygonal.

Season - Rainy season.

Fungal colony light brown with aerial hyphae; mycelium closely interwoven; hyphal size 9.41 $\pm$ 0.27 µm ; mycorrhiza white and dichotomously branched with smooth texture.

Discussion

Among the test nutrient media, MMN's medium was found most suitable to promote growth of the fungi at pH 5.5 (Appendix II), which was followed by Hagem's medium at pH range 5.5-6. The probable reason for the better growth of the symbiont on these media may be attributed to the easily available carbon source and vitamin in the media alongwith the acidophilic nature of the ectomycorrhizal fungi (Melin, 1924, 1925; Modess, 1941; Theodorou and Bowen, 1969). The pH of other nutrient media was higher alongwith complex energy source which might have restricted the growth of the mycobiont. Presence of diammonium orthophosphate in MMN medium might also have favoured the growth of ectomycorrhizal fungi as they readily absorbed nitrogen and phosphorus in ammonium and phosphate form respectively (Harley, 1969). Ferric chloride in MMN and Hagem media has worked as a good co-factor for metabolic activities of the fungi resulting into better growth.

Isolation and growth of mycobiont from mycorrhizal roots were either slow or difficult than from sporocarps (Chu-chou and Grace, 1981). Workers from other parts of the world have also tried to isolate the symbiont from the mycorrhizal roots but little success has been achieved (Chu-chou and Grace, 1988).

Production of mass culture of the symbiont required for large scale inoculation in afforestation programme poses certain problems. In present investigation, the development and multiplication of fungal mycelium were faster in vegetative growth phase of the symbiont. Mikola (1973), Trappe (1977) and Marx (1980) have also supported the findings that mycelial culture was better than spore culture. Fast growing fungi could produce more mycelial inoculum in the short span of time. Sharma and Mishra (1988) were of the opinion that these symbionts may be successful colonizers in early phase of seedling growth than the slow growers,

which may result into the improved growth of seedlings (Kormanik et al., 1977; Malloch et al., 1980). However, pure mycelial inoculum has limited application (Sharma and Mishra, 1988). Some workers have tried to multiply and mass culture the symbionts on grains (Park, 1969; Raman, 1988). They, however, could not get the success for all the isolates. It may, therefore, be suggested that the specific nutrient requirement to the mycobiont may regulate their growth. Therefore, a comprehensive study on the physiology and biochemistry of the fungal symbiont is necessary to provide an aid to improve their growth.

Table 2.1 : Source, place of collection and host of the ectomycorrhizal fungi

Ectomycorrhizal fungi	Source of Isolation	Place of collection	Host
Pisolithus tinctorius	Sporocarp	Riat Khwan Reserve Forest	*Pinus kesiya*
Cenococcum sp.	Mycorrhizal root	Riat Khwan Reserve Forest	*Pinus kesiya*
Scleroderma aurantium	Sporocarp	Naya Bunglow Social Forest	*Pinus kesiya*
Boletus edulis	Sporocarp	Naya Bunglow Social Forest	*Pinus kesiya*
Suillus sp.	Sporocarp	Naya Bunglow Social Forest	*Pinus kesiy*

Table 2.2 : Morphological characters of mycorrhizae formed by different ectomycorrhizal fungi

Ectomycorrhizal fungi	Morphology of mycorrhizae	Colour	Texture
Pisolithus tinctorius	Dichotomously branched	Brownish	Smooth
Cenococcum sp.	Digitate	Black	Rough
Scleroderma aurantium	Dichotomously branched	White	Smooth
Boletus edulis	Coralloid	Creamy	Smooth
Suillus sp.	Dichotomously branched	White	Smooth

Plate - I : Morphology of the ectomycorrhizae formed by :

I - *P. tinctorius*

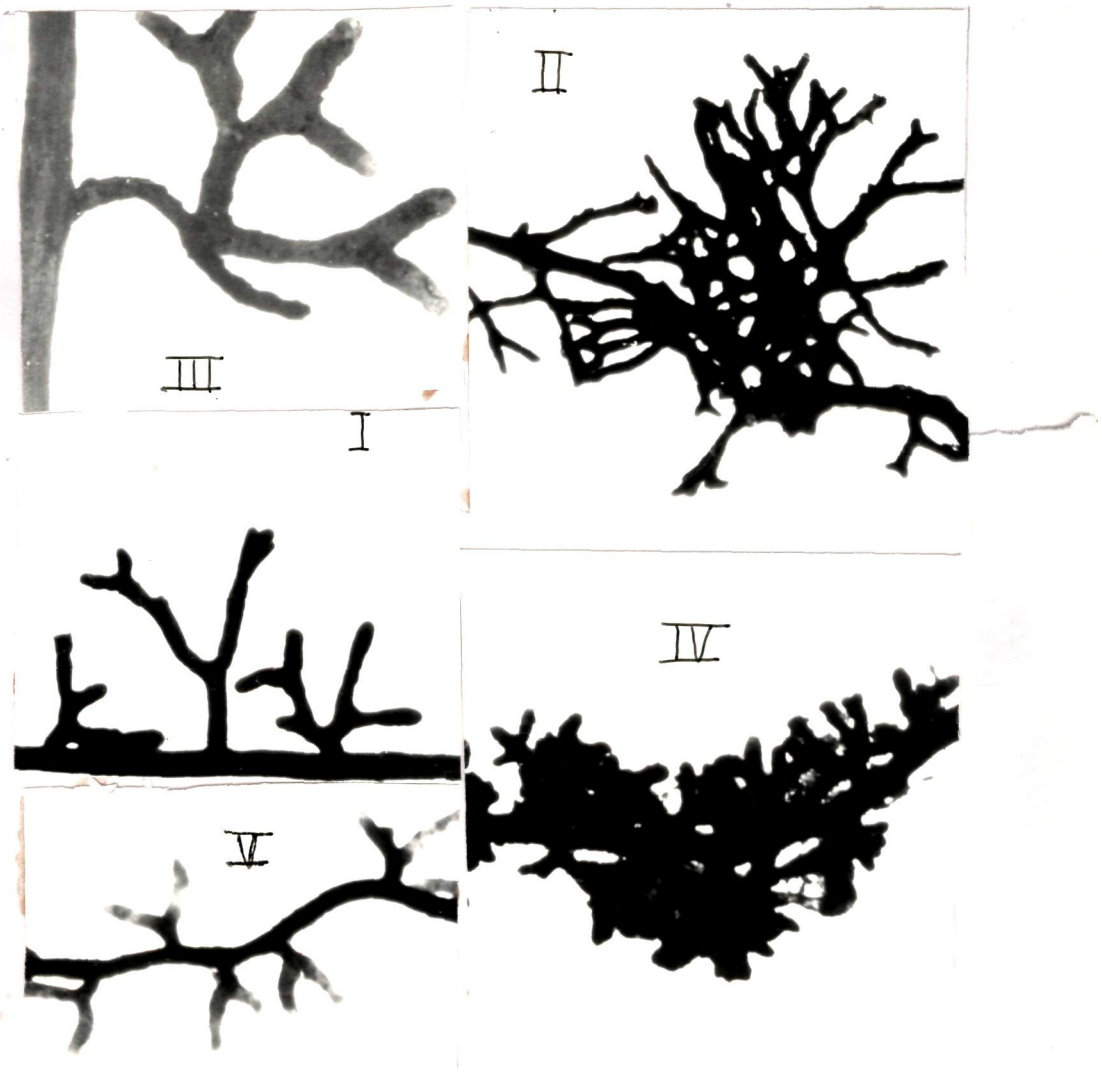
II - *Cenococcum* sp.

III - *S. aurantium*

IV - *B. edulis*

V - *Suillus* sp.

PLATE -1



CHAPTER - III

EFFECT OF CLIMATIC FACTORS ON THE GROWTH BEHAVIOUR OF ECTOMYCORRHIZAL FUNGI IN VITRO CONDITIONS

Introduction

Several criteria have been used to select the ectomycorrhizal fungi for their use in afforestation/plantation programmes under natural conditions (Trappe, 1977). Besides edaphic characters, some climatic factors like temperature, relative humidity and light intensity may also affect the efficiency and growth of the symbionts. It is, therefore, imperative to test an isolate for its adaptability to various climatic conditions available on planting site.

Some experiments on the effect of pH of the medium on ectomycorrhizal fungi have indicated about their optimal growth from 4.5 to 5.5 pH levels (Hung and Trappe, 1983). Besides, some other factors like growth period (Modess, 1941; Norkrans, 1950), nitrogen source (How, 1940), salts concentration (Norkrans, 1950) and aeration (Harley, 1969) may also affect the growth of symbionts.

Similarly, the temperature may influence the growth of ectomycorrhizal fungi in culture conditions (Hacskaylo et al., 1965; Harley, 1969). Reports on the growth of the mycobionts suggest that they have a broad temperature spectrum (Norkrans, 1949; Moser, 1956 and 1958). Their metabolic reaction may also vary at different temperatures (Hacskaylo, 1965).

Light is an important factor for the growth of ectomycorrhizal fungi in symbiotic conditions (Harley, 1969; Harley and Smith, 1983). However, the role of different intensities of light on the efficiency and growth of mycobiont in free culture has less been studied.

Other climatic factors, like relative humidity of the planting site may also influence the germination of spores and the growth of mycelium in free and in symbiotic conditions. Bowen (1964) reported better growth of some fungi at higher and of some at lower relative humidity. Therefore, it is important to evaluate the most suitable conditions required for the optimum growth of the fungi.

Ectomycorrhizal fungi enhance phosphate uptake in host plants through their phosphatase enzymes which hydrolyse complex organic phosphates into available form (Bowen, 1973; Ho and Zak, 1979). The efficiency of phosphate uptake may vary from species to species due to their ability to produce phosphatase enzyme (Ho and Zak, 1979). Interspecific and intraspecific variation in phosphatase activity may therefore be influenced by certain physico-chemical nature of the substratum.

The present study was, therefore, planned to study the effect of various physical factors like pH, temperature, humidity and light intensity on the growth of ectomycorrhizal fungi and their efficiency to produce phosphatase on similar nutrient constituents under free cell culture condition.

Material and Methods

Selection of mycobiont :

Four fast growing ectomycorrhizal fungi, namely, Laccaria laccata, Collybia radicata, Rhizopogon luteolus and Pisolithus tinctorius were selected for the present experiment. The fungi were recultured on MMN nutrient solid medium for 25 days. To study the effect of different factors on the growth of fungi and phospha-

tase activity the liquid and solid MMN nutrient media were used.

(i) Effect of physical factors :

(a) Temperature :

Four temperatures, i.e., 10, 20, 25 and 30°C were selected depending upon the range of temperature prevailing in the natural conditions and were maintained separately in the BOD incubator. Twenty ml of MMN agar medium (pH 5.5) was poured in sterilized petriplate and allowed to solidify. A block of 5 mm diameter was cut from the culture of each fungus grown earlier on MMN solid medium and one such block was inoculated on MMN solid medium in 5 replicates separately. Similarly, one block of 5 mm of each fungus was inoculated in the sterilized 50 ml MMN liquid medium in conical flask (cap. 250 ml) in five replicates separately. The inoculated plates and flasks were incubated at four selected temperatures.

Growth of colony of each fungus was assessed by measuring its diameter at 24 hrs interval upto 168 hrs. The fungal mat growing in conical flask was filtered through Whatman filter paper (No. 1) at an interval of 7 days upto 28 days. The filtered fungal mat was dried at 60°C for 24 hrs in hot air oven and thereafter reweighed on an electrical balance (cap. .01 mg). pH of the liquid culture medium was measured by digital pH meter on each harvest.

(b) Humidity :

Two humidity levels, viz., high (75-85%) and low (45-55%) were maintained inside the humidity incubator chamber at a constant temperature (25°C). To maintain the levels of humidity the water in trays was kept at the bottom inside the BOD incubator and checked regularly. Low level of humidity was maintained by keeping fused $CaCl_2$ in dessicator inside the growth chamber. The $CaCl_2$ was changed everyday. Relative humidity was measured with the help of hygrometer

regularly. The colony growth of fungi was measured at 24 hrs interval and the dry weight was obtained at 7 days interval as mentioned earlier.

(c) Light intensity :

Four light intensities viz., 0, 500, 1000, and 1500 lux were maintained inside the BOD incubators and light was given for 12 hrs photoperiod. In each case colony diameter and dry weight of fungi was determined separately at the regular interval.

(ii) Effect of pH :

Five levels of pH, viz., 3, 5, 6, 7 and 8 were maintained in MMN medium with the help of 0.1N H_2SO_4 and 0.1N NaOH solutions. At pH 3 the medium could not solidify so a semi solid state was considered for the study and care was taken during handling. The inoculated plates and flasks were incubated at 25°C. Growth of colony and dry weight of fungi were assessed as outlined above.

(iii) Phosphatase activity :

Phosphatase activity of each fungus was measured after 14, 21 and 28 days of their incubation under differnt cultural conditions.

Ho and Zak's (1979) method was followed for determining the acid and alkaline phosphatase activity. The amount of P-nitrophenol converted by the ecto-mycorrhizal fungi from P-nitrophenyl phosphate was measured spectrophotometrically. The colony of each fungus was filtered, washed and placed in a sterilized 4 ml of universal buffer solution in a conical flask (cap. 50 ml) separately. 1 ml of 0.115 M disodium P-nitrophenyl phosphate tetrahydrate was added into the universal buffer and the flasks were incubated at 35°C in water bath for 1 hr. After incubation, 4 ml of 0.5 M NaOH was added. The fungal tissue was then filtered through Whatman filter paper (No.42) and dried at 60°C for 24 hrs and

weighed. The enzyme activity was determined from the amount of P-nitrophenol (PNP) in the filtrate. It was measured by Hitachi spectrophotometer (Japan) at 400-420 nm and converted with the standard solution of PNP. The results were represented as micromoles of P-nitrophenol liberated per gram dry weight per hour at 35°C.

Results

Growth of symbionts :

(i) Effect of pH :

Maximum colony growth was shown by Laccaria laccata at pH-5, while its dry weight was maximum at pH-6. Collybia radicata and Rhizopogon luteolus produced maximum colony diameter at pH-6. Pisolithus tinctorius exhibited better colony spread at pH-7 and dry weight at pH-6. Dry weight of L. laccata and C. radicata was lowest in highly acidic and highly alkaline conditions respectively (Table 3.1) whereas, R. luteolus grew well at pH-3 but it was adversely affected by alkaline condition (pH-8). No growth was obtained by P. tinctorius at pH-3 (Fig. 3.1). Production of dry weight by L. laccata, and P. tinctorius was less in highly acidic condition and by R. luteolus and C. radicata in alkaline condition (Table 3.1). Significant increase in the growth of L. laccata was observed at pH-5 (Table 3.5) and by P. tinctorius at pH-6 (Table 3.6). In case of C. radicata and R. luteolus significant increase in growth was obtained at pH-6 (Table 3.5 and 3.6) while P. tinctorius showed better growth in slightly alkaline condition (Fig. 3.1).

Interspecific variation in growth was noticed at different pH levels of medium. C. radicata and R. luteolus exhibited a broad tolerance range for pH, while L. laccata and P. tinctorius were with narrow ecological amplitude and showed

some affinity with moderate acidic and alkaline conditions respectively (Fig. 3.1 and Table 3.1). The fungi also reduced the initial pH of the medium (Table 3.13).

(ii) Effect of temperature :

Maximum colony spread of L. laccata and C. radicata was observed at 25°C, however, R. luteolus produced its maximum growth at 20°C (Fig. 3.2 and Table 3.2).

P. tinctorius did not grow at 10°C on solid medium but could produce very less dry weight on liquid medium. On the other hand, the growth of P. tinctorius and R. luteolus was favoured at 30°C (Table 3.2) compared to other fungi. L. laccata and C. radicata did not grow at 30°C (Fig. 3.2) while other symbionts exhibited slow growth at this temperature (Table 3.2). L. laccata, R. luteolus and P. tinctorius did not show radial growth at 10°C (Fig. 3.2) but their growth was significantly increased at pH 20°C and 25°C (Table 3.7).

C. radicata and P. tinctorius were found adaptive to moderately low and high temperatures respectively (Fig. 3.2). At various temperature the test fungi reduced the pH of the medium (Table 3.14).

(iii) Effect of relative humidity :

High humidity level favoured the growth of most of the fungi on solid medium except P. tinctorius (Fig. 3.3). Maximum colony diameter was attained by L. laccata followed by C. radicata and R. luteolus at this level. P. tinctorius was favoured by the low level of humidity where it produced better colony diameter compared to high level humidity (Fig. 3.3).

In the first harvest R. luteolus and L. laccata produced slightly more dry weight at low level of humidity compared to high level of humidity (Table 3.3). After the first harvest, production of dry matter exhibited increased rate significantly at high humidity level except in the case of P. tinctorius which was

favoured by low level of humidity (Table 3.10). R. luteolus produced maximum dry weight compared to other test fungi (Table 3.3). Reduction in pH of the medium was more at high compared to low level of humidity (Table 3.15).

(iv) Effect of light :

Dark condition favoured the growth of all the ectomycorrhizal fungi. Radial growth as well as dry matter production by the fungi decreased with the increase in intensity of light (Table 3.4 and Fig. 3.4) except in the case of R. luteolus where 500 lux was found more favourable than dark conditions (Fig. 3.4). L. laccata obtained maximum diameter followed by C. radicata and R. luteolus while P. tinctorius showed minimum colony spread at 0 lux light intensity. Maximum dry matter was produced by R. luteolus at different light intensity. It was followed by C. radicata and L. laccata. P. tinctorius produced minimum dry matter (Table 3.4). The rate of reduction in pH of the medium by different fungi also decreased with the increase in intensity (Table 3.16).

(v) Phosphatase activity :

Ectomycorrhizal fungi produced more acid phosphatase activity compared to alkaline phosphatase activity (Table 3.17-3.20).

Acid phosphatase activity was also affected by humidity levels, pH and temperature (Table 3.17-3.20).

Interspecific variation in production of acid phosphatase was also pronounced (Table 3.17-3.20).

Discussion

It has been observed that most of the fungi grew better at pH 5-6. Melin (1924, 1925), Theodorou and Bowen (1969) and Modess (1941) had also advocated

the acidophilic nature of ectomycorrhizal fungi. However, better growth of P. tinctorius at higher pH indicated its capacity to utilise ions in alkaline condition. Bokor (1959) has also reported that some ectomycorrhizal fungi may also grow better in alkaline conditions. Such nature of selectivity of these symbionts can be attributed to their wide range of tolerance. Growth of R. luteolus in extreme acidic conditions may again be correlated to its capability to utilise the ions more efficiently for its metabolic activity (Hung and Trappe, 1983). The selectivity in ion uptake of ectomycorrhizal fungi led to the inter specific or intraspecific variations. Production of organic acids by the mycelium of certain fungi may account to their variability in growth at different pH levels (Hung and Trappe, 1983). These organic acids may help in increasing the uptake of phosphorus either through chelating effect or enhancing the phosphatase activity (Johnston, 1959; Smith, 1980). Such specificity of the mycobiont in ion uptake could be exploited to induce the tolerance in tree species growing under acidic/alkaline soils (Clements et al., 1977).

Better growth in most of the fungi at 25°C could be attributed to their improved metabolic activity. The findings of Harley (1969) have also supported that the optimum temperature for most of the fungi was around 25°C. P. tinctorius grew better at 30°C indicating its optimum temperature. Hacskaylo et al. (1965) suggested that the metabolic reaction varied between the strains of the species and among the various species at different temperatures. The variation in temperature requirement of ectomycorrhizal fungi may be due to adaptive nature of fungal mycelium to different temperatures. Moser (1958), has, however, pointed that fungi growing in montane regions may withstand a freezing temperature. The higher temperature may increase the metabolic activity.

High relative humidity favoured the growth of ectomycorrhizal fungi (Bowen, 1946b). Bhaumik and Clark (1947), Miller and Johnson (1964) and Rovira (1953),

showed that a moderate moisture level was needed for the germination of the spores of the fungi in the soil. Harley (1969) has, however, expressed that physiological activities of the ectomycorrhizal fungi were greatly dependent on adequate oxygen supply. Better growth of ectomycorrhizal fungi at the high level of humidity can be attributed to the sufficient amount of water in the form of water vapour and adequate oxygen to the ectomycorrhizal fungi.

Growth of fungi was decreased by the increase in light intensity. Bjorkman (1942) had the opinion that mycorrhizal fungi are dependent upon simple carbohydrate in culture media, which was attributed to the effect of light. The quantity of sugars in the tissue increased with the increase in light intensity and so encouraged the growth of the fungi. In laboratory condition, fungi get sufficient carbohydrate from the culture medium so they do not need light exposure.Therefore, their growth may be inhibited by the increased light intensity.

Acid phosphatase activity differed under different climatic conditions and also among different ectomycorrhizal fungi. The activity of the fungi was maximum at pH-3, confirming their acidophilic nature (Theodorou and Bowen, 1969; Modess, 1941). The fungi might have produced more acid phosphatase in acidic conditions.

Similarly highest rate of acid phosphatase at 30°C was inversely correlated to the growth of fungi. The lower and higher temperatures were favourable for phosphatase activity, which resulted in the reduced growth. This suggested that most of the fungi could hydrolyse phosphate ions better and more efficiently at higher temperatures (Harley, 1969).

More acid phosphatase activity by the mycobionts at high humidity level can not be attributed to their improved growth at high humid conditions.

Interspecific variation in acid phosphatase activity has suggested that the differences could be correlated to the phosphorus uptake of the ectomycorrhizal

fungi. Similar differences in phosphorus uptake efficiency of some fungi have been reported by Ho and Zak (1979).

Maximum phosphatase activity by P. tinctorius, might be attributed to its eco-physiological adaptability to a broad range of acidic condition.

Based on the investigation carried out, it may be concluded that R. luteolus and P. tinctorius are suitable for inoculation to pine seedlings growing under acidic and alkaline conditions respectively. C. radicata and P. tinctorius can be more efficient at moderately low and high temperatures respectively. P. tinctorius, due to its high phosphatase activity, would be more efficient for phosphorus uptake in low fertilized soil than L. laccata and C. radicata.

Table 3.1 : Effect of different pH on the production of dry weight (mg) by different ectomycorrhizal fungi

pH \ Days	L. laccata				C. radicata				R. luteolus				P. tinctorius			
	7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28
3	4	24	62	86	12.5	42	125	134	27	146	206	223	1	1	1	1.5
5	71	155	163	186	45.5	71	169	213	133	298	336	368	1	1	1	10
6	23	77	186	205	62.5	83	229	288	138	272	322	391	4	78	154	179
7	20	27	71	101	37	73	100	129	29	130	229	321	3	79	96	121
8	10	15	60	95	37	62	72	102	32	64	93	103	2	28	31	43.5

Table 3.2 : Effect of different temperature on the production of dry weight (mg) by different ectomycorrhizal fungi

	L. laccata				*C. radicata*				*R. luteolus*				*P. tinctorius*			
Days / Temp. (°C)	7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28
10	33	44	49	55	19	64	66	67	21	101	105	110	6	16	52	57
20	32	322	401	420	36	387	422	432	106	399	638	640	16	24	31	32
25	31	356	386	411	20	399	402	410	39	335	477	481	34	36	52	60
30	19	68	128	132	11	12	48	50	18	47	790	800	6	40	153	162

Table 3.3 : Effect of relative humidity (%) on the production of dry weight (mg) by different ectomycorrhizal fungi

	L. laccata				*C. radicata*				*R. luteolus*				*P. tinctorius*			
Days	7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28
RH* (%)																
High level**	31	356	386	411	20	399	402	410	39	335	477	481	34	36	52	60
Low level***	42	56	96	114	18	42	82	134	65	121	178	195	34	55	76	103

* = Relative Humidity

** = 75-85%

*** = 45-55%

Table 3.4 : Effect of different light intensities on the production of dry weight (mg) by different ectomycorrhizal fungi

	L. laccata				C. radicata				R. luteolus				P. tinctorius			
Days / Lux	7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28
0	31	386	411	434	43	399	410	416	39	335	477	481	34	36	52	60
500	20	156	233	308	23	94	299	379	31	206	356	395	21	31	44	50
1000	21	78	240	250	24	143	268	284	34	198	305	380	21	32	38	46
1500	13	55	110	140	14	80	112	135	20	108	195	225	11	19	31	34

Table 3.5 : Analysis of variance for the effect of pH on the radial growth of ectomycorrhizal fungi

Source of variance	SS	df	MSS	Calculated value	F value at 5%
Effect of pH	4196.29	4	1049.07	32.1*	2.5
Effect of species	9603.78	3	3201.26	98.1*	2.74
Effect of days	15045.12	6	2507.52	76.8*	2.23
Effect of pH x species	6772.66	12	564.38	17.3*	1.90
Effect of pH x days	1331.16	24	52.46	1.7*	1.67
Effect of species x days	3443.41	18	191.30	5.7*	1.76
Error	2349.74	72	32.63	0	-
Total	42742.20	139	307.49	0	-

* Significant at P = 0.05

Table 3.6 : Analysis of variance for the effect of pH on the dry weight production by ectomycorrhizal fungi

Source of variance	SS	df	MSS	Calculated value	F value at 5 %
Effect of pH	147500.05	4	36875.00	52.7*	2.65
Effect of species	245477.31	3	81825.77	116.8*	2.89
Effect of days	196400.38	3	65466.79	93.5*	2.89
Effect of pH x species	77525.09	12	6460.42	9.2*	2.03
Effect of pH x days	30244.13	12	2520.34	3.6*	2.03
Effect of species x days	33743.07	9	3749.23	5.4*	2.12
Error	25213.29	36	700.36	0	-
Total	756103.34	79	9570.92	0	-

* Significant at P = 0.05

Table 3.7 : Analysis of variance for the effect of temperature on the radial growth of ectomycorrhizal fungi

Source of variance	SS	df	MSS	Calculated value	F value at 5%
Effect of temperature	8762.25	3	2920.18	81.7*	2.78
Effect of species	2135.61	3	711.87	19.9*	2.78
Effect of days	6982.80	6	1163.80	32.6*	2.28
Effect of temperature x species	3877.05	9	430.78	12.1*	2.10
Effect of temperature x days	3845.46	18	213.63	6.0*	1.80
Effect of species x days	948.22	18	52.68	1.5	1.80
Error	1930.13	54	35.74	0	-
Total	28481.54	111	256.59	0	-

* Significant at P = 0.05

Table 3.8 : Analysis of variance for the effect of temperature on the dry weight production by ectomycorrhizal fungi

Source of variance	SS	df	MSS	Calculated value	F value at 5%
Effect of temperature	522347.76	3	174115.92	24.0*	2.96
Effect of species	222539.62	3	74179.87	10.2*	2.96
Effect of days	340843.79	3	113614.59	15.7*	2.96
Effect of temperature x species	244302.45	9	27144.71	3.8*	2.20
Effect of temperature x days	170372.81	9	18930.31	2.6*	2.20
Effect of species x days	91501.02	9	10166.78	1.4	2.20
Error	195525.06	27	7241.67	0	-
Total	1787432.53	63	28371.94	0	-

* Significant at P = 0.05

Table 3.9 : Analysis of variance for the effect of humidity on the radial growth of ectomycorrhizal fungi

Source of variance	SS	df	MSS	Calculated value	F value at 5%
Effect of humidity	538.18	1	538.18	23.6*	4.41
Effect of species	2314.66	3	771.55	33.8*	3.16
Effect of days	8994.29	6	1499.04	65.7*	2.66
Effect of humidity x species	1031.98	3	343.99	15.1*	3.16
Effect of humidity x days	431.91	6	71.98	3.2*	2.66
Effect of species x days	1614.47	18	89.69	3.9*	2.24
Error	410.74	18	22.82	0	-
Total	15336.27	55	278.84	0	-

* Significant at P = 0.05

Table 3.10 : Analysis of variance for the effect of humidity on the dry weight production by ectomycorrhizal fungi

Source of variance	SS	df	MSS	Calculated value	F value at 5%
Effect of humidity	198137.06	1	198137.06	50.9*	5.12
Effect of species	143324.25	3	47774.74	12.3*	3.86
Effect of days	201332.51	3	67110.83	17.2*	3.86
Effect of humidity x species	87403.69	3	29134.56	7.5*	3.86
Effect of humidity x days	73888.71	3	24629.52	6.3*	3.86
Effect of species x days	49106.18	9	5456.24	1.4	3.18
Error	35027.62	9	3891.95	0	-
Total	788220.01	31	25426.43	0	-

* Significant at P = 0.05

Table 3.12 : Analysis of variance for the effect of light intensities on the dry weight production by ectomycorrhizal fungi

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of light	239260.750	3	79753.583	124.01*	2.96
Effect of species	370038.875	3	123346.292	191.793*	2.96
Effect of days	491828.625	3	163942.875	254.917*	2.96
Effect of light x species	66033.375	9	7337.042	11.408*	2.20
Effect of light x days	75987.125	9	8443.014	13.128*	2.20
Effect of species x days	138887.500	9	15431.944	23.995*	2.20
Error	50516.750	27	643.123	0	-
Total	1422553.000	63	22580.206	0	-

* Significant at P = 0.05

Table 3.13 : Change in pH in the culture filtrates of the ectomycorrhizal fungi grown in different pH

pH \ Days	L. laccata				C. radicata				R. luteolus				P. tinctorius			
	7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28
3	3.2	2.8	2.0	1.9	3.2	2.5	2.0	2.3	3.1	1.9	1.5	1.7	3.5	2.5	2.1	2.3
5	5.9	3.9	2.6	3.0	5.3	5.1	2.6	2.7	2.9	2.5	1.9	2.1	5.6	5.0	3.4	4.3
6	5.6	4.4	2.1	2.0	5.8	4.3	3.1	2.9	3.7	2.0	2.2	2.3	5.4	2.8	2.1	2.0
7	5.2	5.2	5.0	4.9	5.0	5.5	4.4	2.9	5.0	5.2	4.9	2.8	5.1	4.8	3.6	3.4
8	5.0	5.8	5.8	5.7	4.5	3.3	5.9	3.3	5.2	5.5	6.8	3.3	5.4	6.3	5.7	5.5

Table 3.14 : Change in pH in the culture filtrates of the ectomycorrhizal fungi grown at different temperature

	L. laccata				C. radicata				R. luteolus				P. tinctorius			
Days / Temp. (°C)	7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28
10	4.5	3.7	3.9	3.6	5.5	5.9	4.5	5.9	5.7	4.0	4.0	4.6	5.7	5.8	3.8	5.9
20	4.3	3.7	3.3	3.3	5.2	4.9	4.0	4.0	3.2	3.7	3.8	3.9	4.0	4.8	5.3	4.4
25	4.6	3.7	3.1	3.2	4.1	4.7	4.8	3.8	3.9	3.7	3.7	2.9	5.7	4.5	4.9	3.4
30	3.3	3.6	4.2	3.6	3.3	4.6	6.1	4.3	3.3	4.1	4.9	4.1	3.2	4.2	4.1	3.3

Table 3.15 : Change in pH in the culture filtrates of the ectomycorrhizal fungi grown at different relative humidity (%)

	L. laccata				*C. radicata*				*R. luteolus*				*P. tinctorius*			
Days RH* (%)	7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28
High level**	4.6	3.7	3.1	3.1	4.1	4.8	4.8	3.8	3.9	3.7	3.7	2.9	5.7	4.5	4.9	3.4
Low level***	5.1	5.0	4.6	3.8	5.0	6.0	5.6	5.6	4.4	4.0	3.5	3.8	4.1	4.5	5.2	3.5

* = Relative Humidity

** = 75-85%

*** = 45-55%

Table 3.16 : Change in pH in the culture filtrates of the ectomycorrhizal fungi grown at different intensities of light

	L. laccata				C. radicata				R. luteolus				P. tinctorius			
Days / Lux	7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28
0	4.6	3.7	3.1	3.1	4.1	4.7	4.8	3.8	3.9	3.7	3.7	2.9	5.7	4.5	4.9	3.4
500	5.2	4.0	4.2	3.4	5.2	4.6	5.3	4.7	3.3	3.7	3.7	3.5	4.0	5.3	4.5	3.4
1000	5.0	4.2	4.4	3.4	5.2	4.7	4.9	4.6	3.7	3.5	4.0	3.6	5.4	5.3	4.8	3.5
1500	5.2	4.9	4.7	5.0	5.5	5.1	4.8	4.9	4.0	4.2	4.0	3.8	5.7	5.6	5.1	4.5

Table 3.17 : Acid and alkaline phosphatase activity (μ mol/g dry wt.) of different ectomycorrhizal fungi at different pH

pH		L. laccata			C. radicata			R. luteolus			P. tinctorius		
		14	21	28	14	21	28	14	21	28	14	21	28
3	AP	78.0	34.3	24.1	96.7	18.9	16.2	16.4	10.0	9.9	35.9	123.6	219.2
	Al.P	29.8	34.3	18.6	9.6	2.1	11.4	6.9	1.5	8.5	14.4	10.8	24.0
5	AP	28.0	12.7	16.4	31.6	7.3	10.0	6.2	7.0	5.9	87.7	319.8	152.9
	Al.P	5.8	3.0	10.4	11.8	1.0	7.6	7.2	1.4	5.3	14.4	251.5	76.0
6	AP	25.2	11.2	10.1	27.5	9.3	7.2	5.8	6.6	5.4	34.9	477.1	24.0
	Al.P	3.8	11.6	10.8	16.7	9.0	7.7	8.4	2.7	4.1	27.7	95.2	17.9
7	AP	125.4	29.7	20.5	29.9	21.5	16.0	17.4	9.4	6.7	25.0	35.4	17.2
	Al.P	14.0	16.9	7.7	4.2	8.0	3.9	16.1	1.4	5.0	14.2	2.6	15.4
8	AP	18.0	37.3	22.1	29.2	11.0	22.6	32.5	18.0	19.6	86.8	40.4	51.8
	Al.P	9.0	7.7	14.7	3.8	5.4	0.9	20.0	3.0	11.1	42.6	1.2	18.2

14, 21 and 28 are the number of days

AP = Acid Phosphatase; Al.P = Alkaline phosphatase

Table 3.18 : Acid and alkaline phosphatase activity (μ mol/g dry wt.) of different ectomycorrhizal fungi at different temperatures

Temp.(°C)		L. laccata			C. radicata			R. luteolus			P. tinctorius		
		14	21	28	14	21	28	14	21	28	14	21	28
10	AP	47.5	41.5	37.0	28.7	31.7	30.2	21.2	20.0	18.5	20.4	11.8	9.4
	Al.P	1.0	1.9	4.7	10.3	9.6	12.5	17.1	5.8	4.3	1.8	0.34	0.9
20	AP	10.2	6.5	6.9	2.5	2.4	2.6	2.6	2.3	3.4	3.6	3.5	2.5
	Al.P	2.9	0.6	1.2	0.5	0.6	1.6	1.2	0.8	1.4	1.2	3.5	0.8
25	AP	10.2	2.6	2.1	12.1	2.5	1.9	11.5	3.5	2.5	9.2	3.5	2.6
	Al.P	1.2	0.5	0.4	3.2	0.4	0.5	2.1	0.9	1.1	1.1	0.2	0.1
30	AP	30.9	16.5	15.8	176.7	32.5	41.4	44.8	2.7	2.6	133.2	12.3	46.5
	Al.P	27.6	16.8	16.4	13.4	26.9	21.0	20.8	1.2	1.0	15.5	5.7	20.0

14, 21 and 28 are the number of days

AP = Acid Phosphatase; Al.P = Alkaline phosphatase

Table 3.19 : Acid and alkaline phosphatase activity (μ mol/g dry wt.) of different ectomycorrhizal fungi at different light intensities

Intensity (lux)		L. laccata			C. radicata			R. luteolus			P. tinctorius		
		14	21	28	14	21	28	14	21	28	14	21	28
0	AP	10.2	2.6	2.1	12.1	2.5	1.9	11.5	3.5	2.5	9.2	3.5	2.6
	Al.P	1.2	1.5	1.4	3.2	0.4	0.5	1.1	0.9	1.1	1.1	1.2	0.1
500	AP	13.0	6.4	5.2	20.3	5.0	4.1	10.0	4.5	4.0	65.4	28.9	33.3
	Al.P	12.3	7.0	6.4	20.0	5.4	1.4	9.4	5.8	4.4	60.5	22.6	40.6
1000	AP	12.5	19.7	20.5	19.9	11.5	15.0	17.4	19.4	16.7	25.0	44.4	17.2
	Al.P	14.0	11.9	7.7	4.2	7.8	3.2	11.1	1.2	5.0	4.2	6.2	4.5
1500	AP	17.0	27.3	22.5	23.2	22.6	12.1	12.5	18.0	19.5	36.8	40.4	45.8
	Al.P	4.0	5.7	7.4	3.8	5.4	2.9	2.4	3.0	7.1	4.6	2.2	8.2

14, 21 and 28 are the number of days

AP = Acid Phosphatase; Al.P = Alkaline phosphatase

Table 3.20 : Acid and alkaline phosphatase activity (µ mol/g dry wt.) of different ectomycorrhizal fungi at different levels of humidity

RH (%)		L. laccata			C. radicata			R. luteolus			P. tinctorius		
		14	21	28	14	21	28	14	21	28	14	21	28
High	AP	41.2	3.9	3.9	35.6	3.7	3.2	20.1	3.2	3.3	45.5	24.7	28.0
	Al.P	7.1	4.2	4.7	2.4	2.8	2.0	6.5	4.1	1.3	15.3	3.3	24.7
Low	AP	36.2	24.6	21.0	45.8	36.2	20.9	16.9	9.8	7.6	36.8	28.5	22.9
	Al.P	9.1	5.2	4.4	15.6	10.1	7.8	9.1	5.7	4.2	25.1	15.2	12.1

14, 21 and 28 are the number of days

AP = Acid Phosphatase; Al.P = Alkaline phosphatase

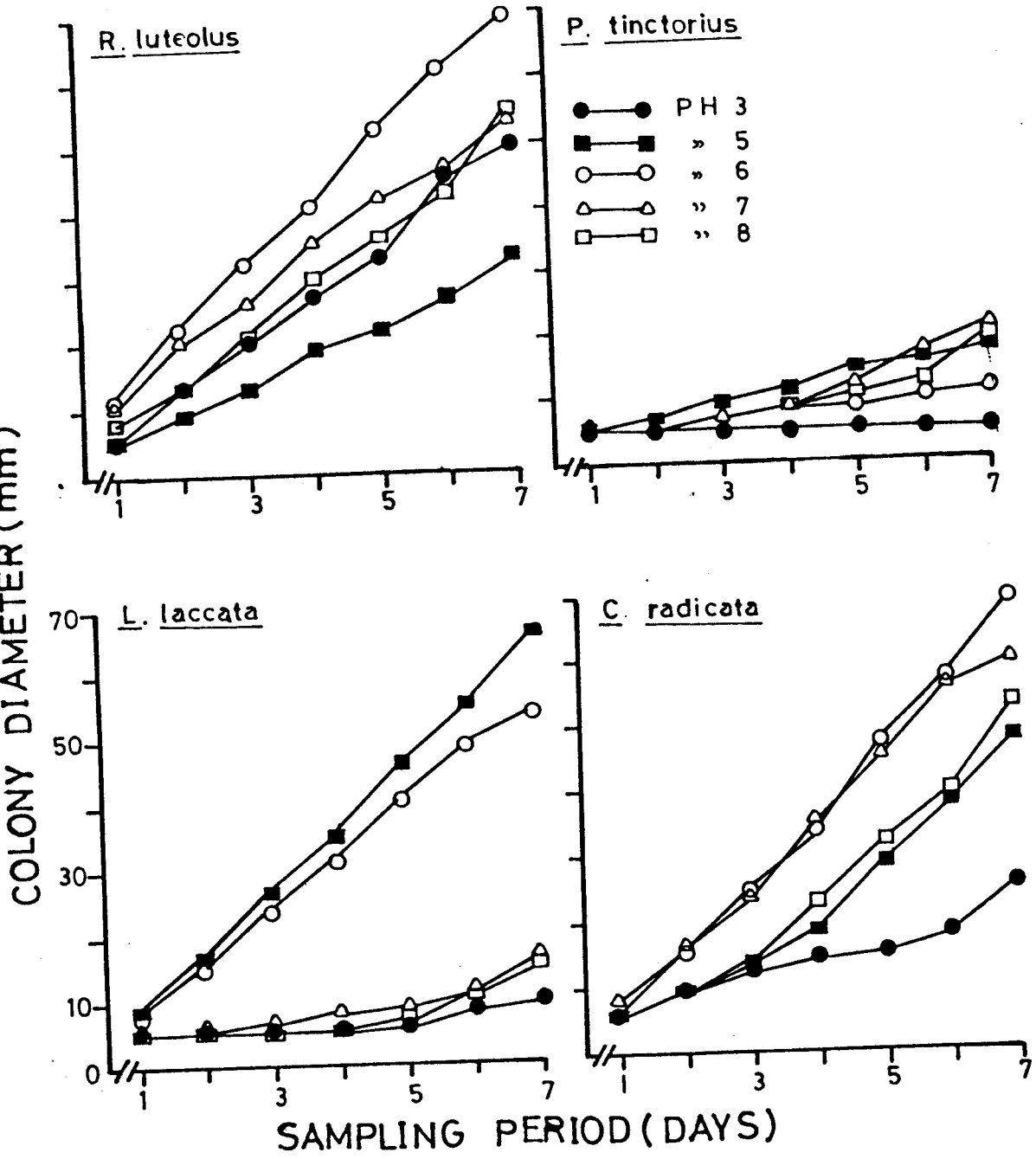


Fig. 3.1 : Radial growth of ectomycorrhizal fungi at different pH.

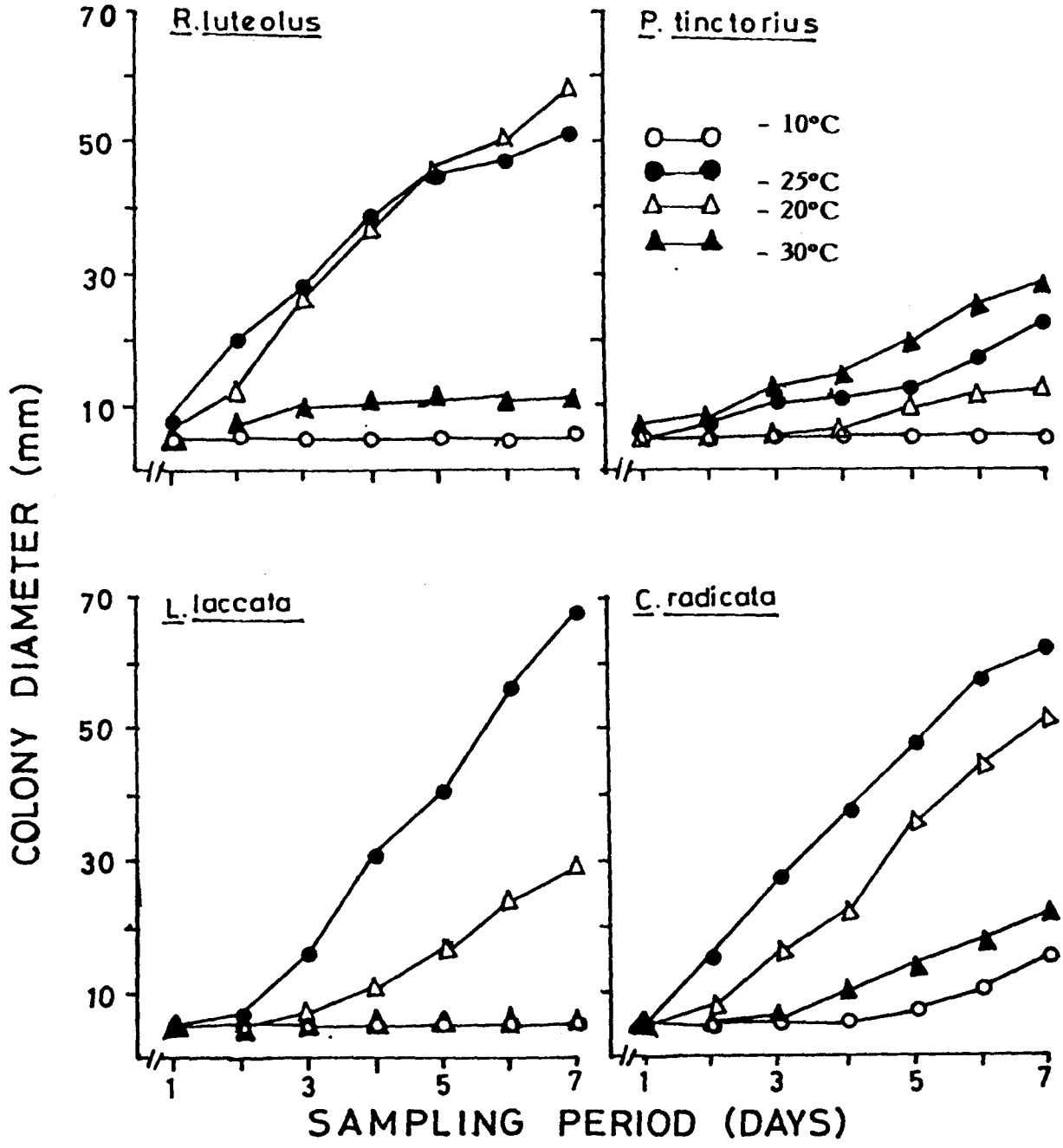


Fig. 3.2 : Radial growth of ectomycorrhizal fungi at different temperatures.

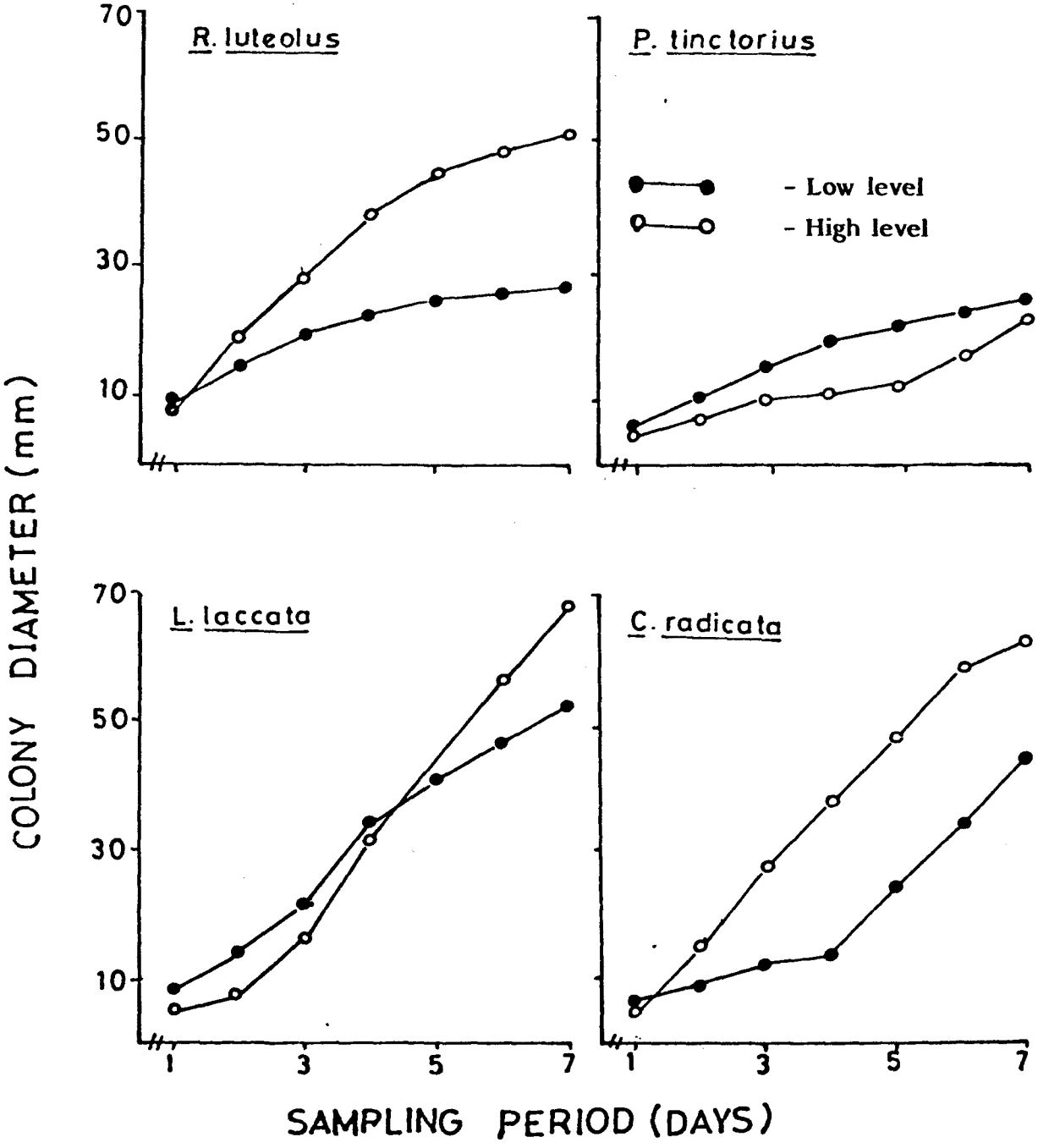


Fig. 3.3 : Radial growth of ectomycorrhizal fungi at different levels of relative humidity (%).

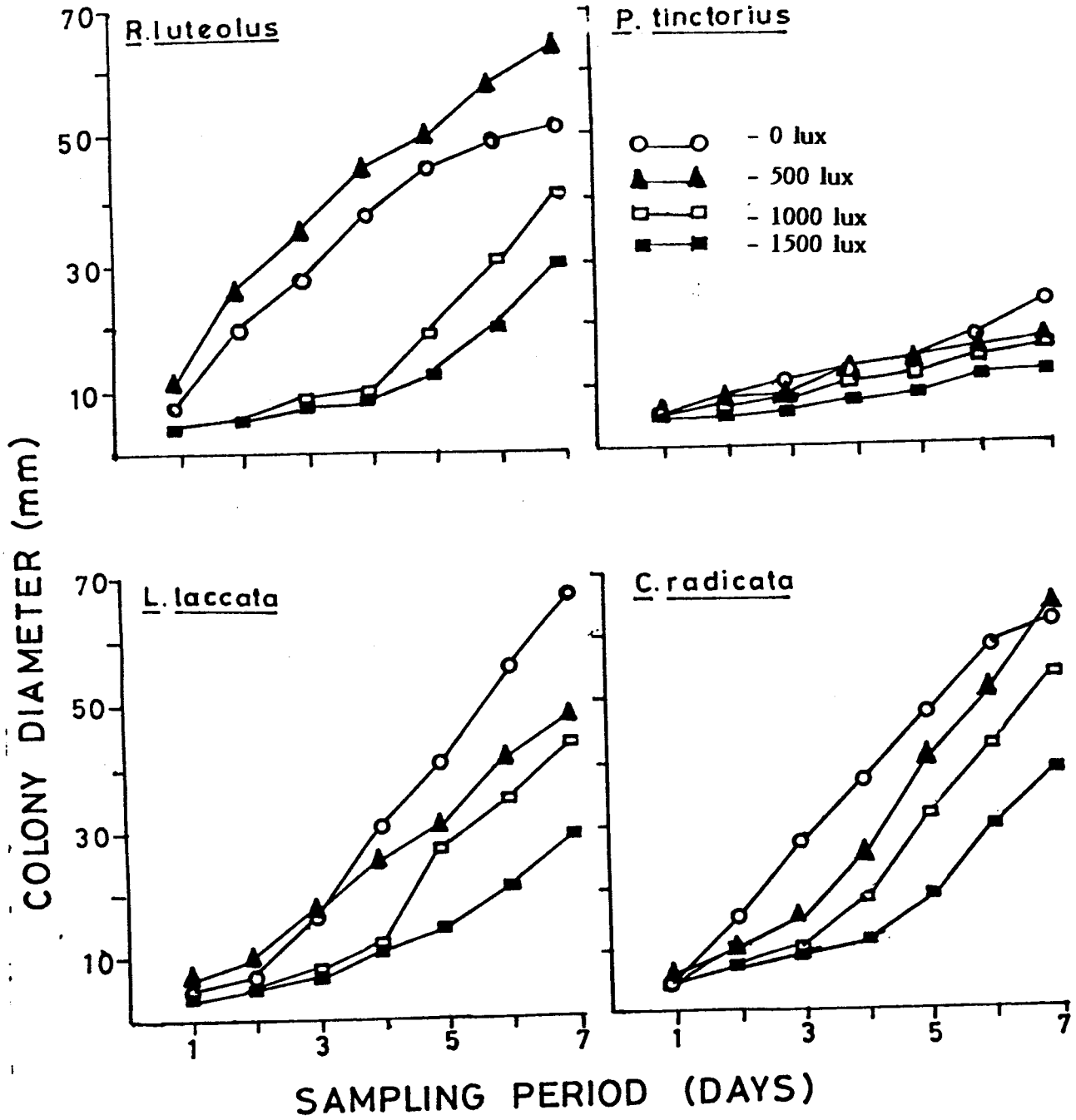


Fig. 3.4 : Radial growth of ectomycorrhizal fungi at different light intensities (lux).

ate - 2 : Effect of pH of the culture medium on the growth of different ectomycorrhizal fungi. ApH_5 - L. laccata at pH-5, BpH_5 - C. radicata at pH-5, CpH_5 - R. luteolus at pH-5 and DpH_8 - P. tinctorius at pH-8.

PLATE -2

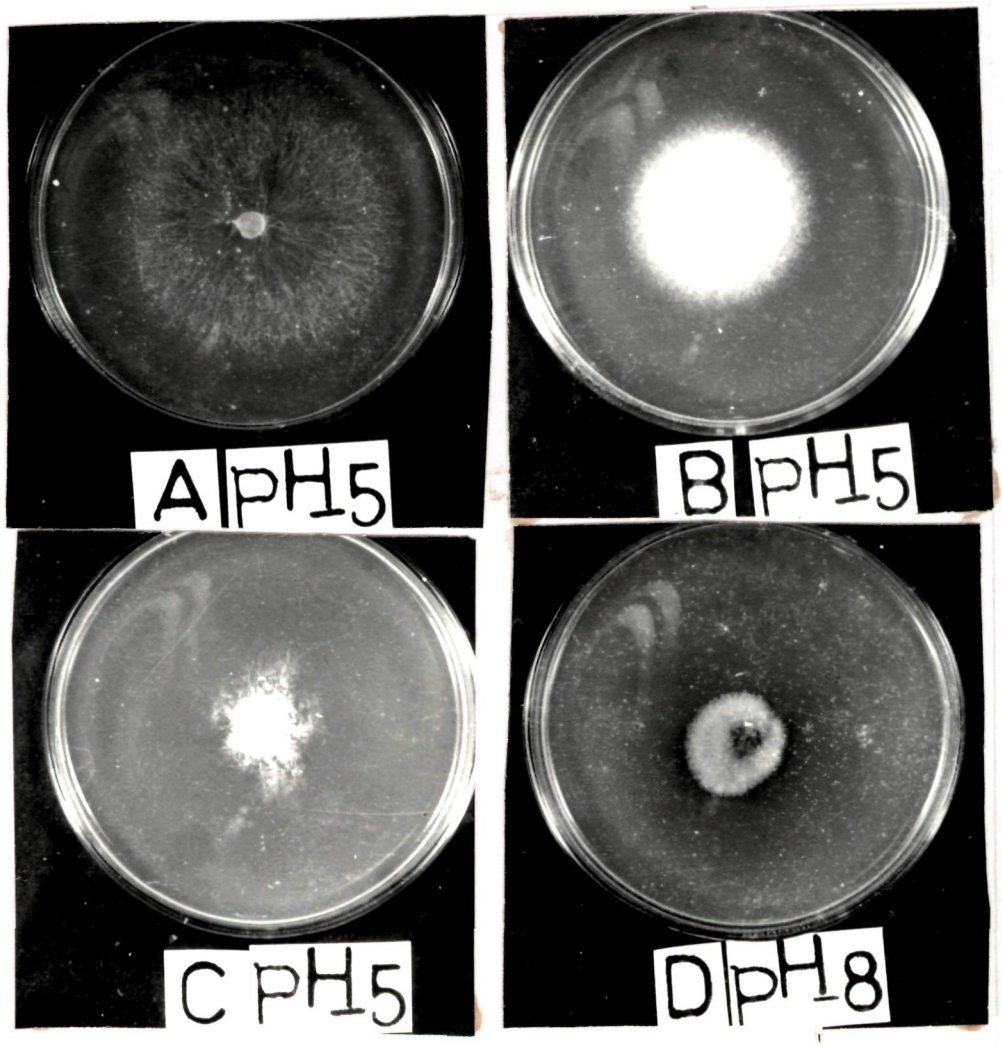
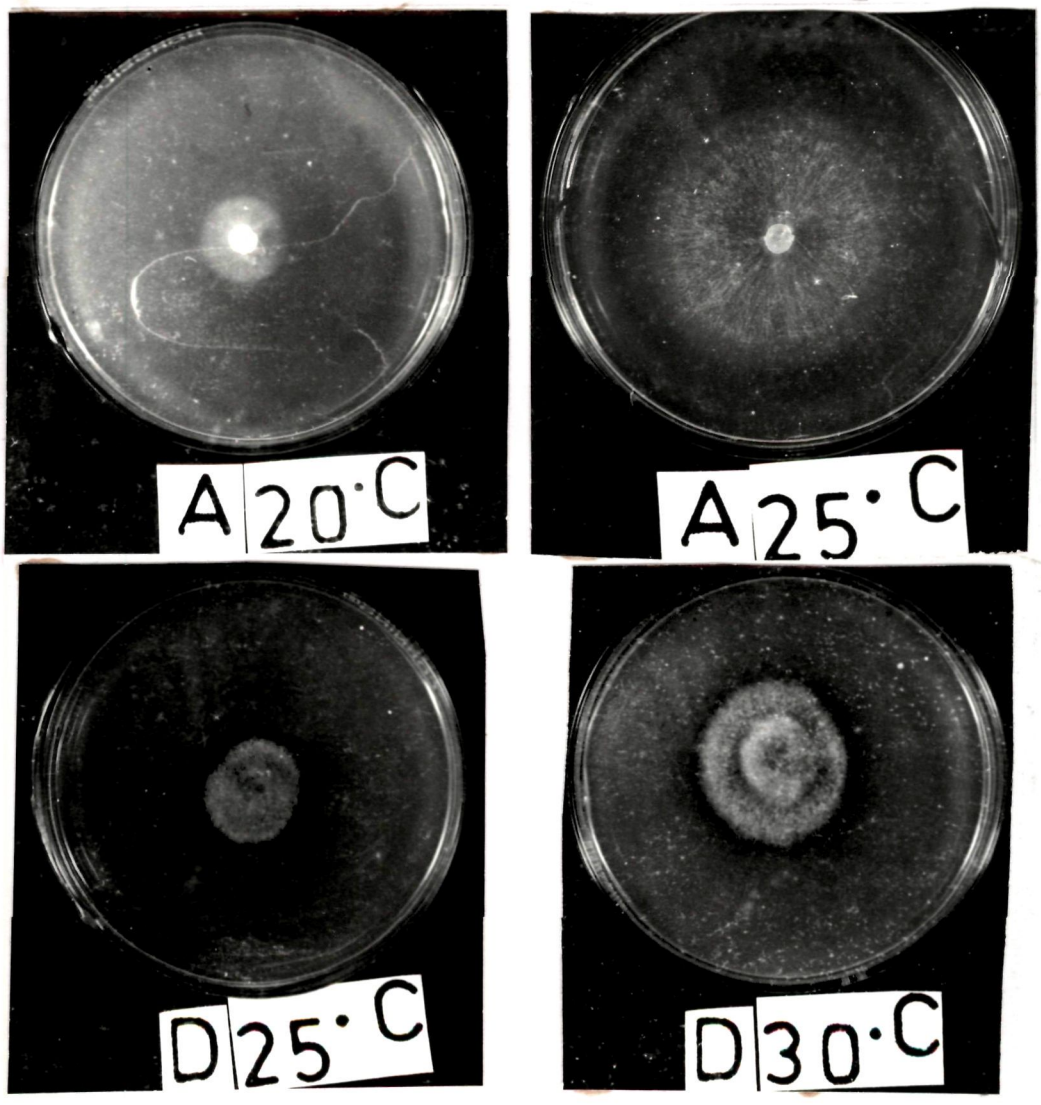


Plate - 3 : Effect of temperature on the growth of different ectomycorcorhizal fungi. A 20°C - L. laccata at 20°C, A 25°C - L. laccata at 25°C, D 25°C - P. tinctorius at 25°C and D 30°C - P. tinctorius at 30°C.

PLATE-3



CHAPTER - IV

EFFECT OF CLIMATIC CONDITIONS ON THE DEVELOPMENT OF ECTOMYCORRHIZAE

Introduction

There have been substantial studies on the edaphic and biotic effects on the development of mycorrhizae. However, investigations on the climatic effects on the mycorrhizal development are very meagre (Bowen and Theodorou, 1973). The intensity of mycorrhizal infection is greatly influenced by the soil macro-elements especially the phosphorus (Hatch, 1937). Climatic factors like light intensity (Hatch, 1937; Bjorkman, 1942), temperature (Theodorou and Bowen, 1971) and humidity may also be important for the development of mycorrhiza.

High light intensity may be favourable for the development of ecto-mycorrhizae (Hatch, 1937, Bjorkman, 1942). However, addition of phosphate at high light intensity was found to reduce the development of mycorrhizae (Harley and Waid, 1955; Hacskaylo and Snow, 1959; Boullard, 1961; Son and Smith, 1988).

Reduction in the frequency of mycorrhizae are reported to be inversely proportional to light intensity (Gast, 1937). On the other hand, higher mycorrhizal frequency at lower light intensity has also been reported by few workers (Mikola, 1948; Harley and Waid, 1955; Son and Smith, 1988). The

reason for reduced mycorrhizae has been assigned to the concentration of phosphorus in the root tissue (Mosse, 1973). However, relationship between the symbionts and the mycorrhizal plants are inter-dependent. The symbionts require additional energy supply from host in the form of carbon compounds for their growth (Pang and Paul, 1980; Snellgrove et al., 1982, 1986; Son and Smith, 1988). Therefore, the light intensity may regulate the photosynthate affecting the development of mycorrhizae. Hence, light is an important factor in addition to nitrogen and phosphorus in influencing the amount of free soluble sugars in the roots which regulates the development of mycorrhizal infection.

Temperature is another factor affecting the colonization of mycorrhizae (Bowen and Theodorou, 1973). Studies have indicated that a temperature range from 16°C to 25°C was most suitable for the development of mycorrhizae (Theodorou and Bowen, 1971). The influence of temperature on the mycorrhiza has mostly been correlated to the root metabolism and root exudates of the host (Bowen, 1970). However, colonization and intensity of different mycorrhizal fungi may vary even at the same temperature (Marx et al., 1970; Theodorou and Bowen, 1971).

Formation of mycorrhiza in pine seedlings can also be regulated by the supply of water (Worley and Hacskaylo, 1959). Moderate to high relative humidity can favour mycorrhizal development (Bowen and Theodorou, 1973).

Some fragmentary informations on the effect of climatic conditions on the development of ectomycorrhizae have been collected. However, correlative studies between environmental factors and the mycorrhizae are not available. Therefore, it was planned to investigate the effect of light, temperature and relative humidity on the development of mycorrhizae and their efficiency on the growth of pine seedlings at different phosphorus

levels in soil.

Material and Methods

Selection of Mycobiont

Four fast growing ectomycorrhizal fungi, selected earlier (Chapter 3 of the thesis), were recultured on MMN nutrient agar medium for 25 days. Mycelial cultures of these fungi were prepared on the MMN liquid medium for their inoculation to pine seedlings under different climatic conditions.

Pine seedlings (P. kesiya) were raised following the method described earlier (Chapter 2).

Garden soil and sand (1:1) was steam sterilized at 15 lb/inch pressure for 60 minutes two times at 24 hrs interval and allowed to cool.

Four doses of available phosphorus (SSP), i.e. 0P, 1P, 1/2P and 2P were prepared considering 30 kg phosphorus/hectare as normal dose. Amount of SSP was added to each pot to get the final level of phosphorus at the following rate :

Levels of phosphorus	Amount of SSP/kg soil
0 level	No addition
Normal (1P)	92.308 mg
Half of the normal (1/2P)	46.153 mg
Double of the normal (2P)	184.615 mg

(i) Effect of light

To study the effect of light intensity three intensities, i.e. 30,000, 10,000 and 500 lux, termed as high, moderate and low light intensities respectively, were maintained in the net house. High, moderate and low light

intensities were provided by keeping the whole set of experimental pots under direct sunlight, under light chamber made by white cheese cloth and under light chamber made by black cloth respectively. Plastic pots (20 cm x 21 cm) were filled by soil:sand mixture at the rate of 8 kg/pot in seven replicates for each fungal species under each light intensity and phosphorus levels separately.

Pine seedlings of 2 cm long radicles were transferred to the pots at the rate of 10 seedlings/pot and inoculated with 50 ml homogenized solution of each fungal mycelia separately. The pots were watered regularly by tap water. Six harvests of seedlings to measure the mycorrhizal development and the growth of pine seedlings were carried out at one month interval.

(ii) Effect of temperature

Three temperatures, viz., 10, 25 and 30°C were maintained inside the growth chambers at 300 lux (approx.) light intensity and 16 hrs photoperiod. Earthen pot (8.5 cm x 9.5 cm) filled with sand:soil mixture (200 g soil/pot) were steam sterilized at 15 lb/inch pressure. Fourteen replicates for each fungal species at each temperature and phosphorus level were maintained separately. Four pine seedlings were transplanted into each pot and allowed to establish under natural conditions for two months. Thereafter, the seedlings were inoculated by 10 ml mycelial homogenate of each mycobiont separately (1:2, w/v) and transferred to different temperatures maintained in growth chambers separately.

Pots were watered with sterilized water regularly. Twelve seedlings were harvested at each harvest at monthly interval.

(iii) Effect of relative humidity

Two levels of relative humidities were maintained inside the growth

chamber, i.e. high humidity level (90% - 100%) and low humidity level (50% - 65%). Four seedlings were transplanted in the earthen pots (diam. 8.5 cm) filled with sterilized soil:sand mixture (1:1) and were inoculated with different mycorrhizal fungi (as detailed above). Seedlings were allowed to grow at two humidity levels at 25°C, 300 lux light intensity and 16 hrs photoperiod. Harvesting of seedlings was done at monthly interval similar to the temperature set.

(iv) Estimation of mycorrhizal development

Development of ectomycorrhizae was estimated following the procedure as outlined by Sharma (1981). Percentage mycorrhizal association was obtained by counting dichotomously branched rootlets at a regular interval. One complete ultimate dichotomy was counted as one mycorrhiza. Percentage mycorrhizal development was estimated as :

$$\text{Mycorrhizal development (\%)} = \frac{\text{Total no. of dichotomously branched rootlets}}{\text{Total rootlets no.}} \times 100$$

(v) Survivorship of the seedlings

Survival of the inoculated and uninoculated pine seedlings was observed for each treatment separately at each harvest with the following formula :

$$\text{Survivorship of the seedlings (\%)} = \frac{\text{Total no. of pine seedlings at the time of harvest}}{\text{Total no. of pine seedlings at the initial time of the experiment}} \times 100$$

(vi) Growth of pine seedlings

The growth of plants was assessed in terms of shoot and root length

at each harvest of both inoculated and controlled seedlings. Number of secondary roots was also counted at each harvest and interpreted with the seedling growth. Fresh and dry weights of shoots and roots were also determined separately at each harvest to assess the growth. Fresh weight of the shoots and roots was taken immediately after the harvest and dry weights of cleared shoots and roots was calculated after cooling the dried matter at 60°C for 24 hrs in the hot air oven.

Results

A. Development of mycorrhiza

(i) Effect of light

High light intensity enhanced the colonization and root length compared to moderate and low light intensities (Tables 4.8 and 4.1). Maximum shoot height of seedlings was obtained at moderate light intensity (Table 4.1). Among the ectomycorrhizal fungi Laccaria laccata promoted maximum shoot as well as root length followed by Rhizopogon luteolus and Collybia radicata at high light intensity. Seedlings with Pisolithus tinctorius attained minimum shoot and root length; (Table 4.1). At moderate light intensity maximum shoot and root lengths were obtained by the seedlings inoculated with P. tinctorius followed by L. laccata and C. radicata. At this light intensity seedlings with R. luteolus attained minimum shoot and root length. Least growth of seedlings was noticed at low light intensity. Variation in shoot and root lengths of seedlings inoculated with different fungi was least (Table 4.1). Profuse growth of needles was also noticed under this light intensity while dense clusters of needles was apparent on the terminal shoot at high light intensity (Plates 6 and 7). Fresh and dry weights of shoot were also higher under moderate light intensity than high light intensity with 2P and 1/2P levels except L. laccata and C. radicata with 2P levels while fresh and dry weights of shoot were found more under high light intensity except

in the case of R. luteolus inoculated seedlings with 1P level (Table 4.4 and 4.2). Reverse trend was observed in the case of root dry weight (Table 4.5 and 4.3). Root and shoot dry matter production were found affected significantly by the harvesting period and different species of ectomycorrhizal fungi at high and moderate light intensities (Tables 4.10-4.13) except root dry matter at high light intensity (Table 4.10). Seedling growth was either invisible or extremely slow under low light intensity (Plates 6 and 7). Mycorrhizal fungi did not colonize the roots of pine under this light intensity (Table 4.8).

Significantly, enhanced growth of pine seedlings was observed with 2P level compared to 1P and 1/2P levels when inoculated with ectomycorrhizal fungi. Relative growth of seedlings with 2P level was higher at moderate light intensity except in the case of L. laccata. Insignificant difference in growth was noticed between 1P and 1/2P levels under moderate light intensity except at high light intensity where 1P level was found more suitable than 1/2P level in promoting the growth of pine seedlings. Seedlings with C. radicata obtained maximum shoot and root lengths with 1P level at high light intensity followed by P. tinctorius and L. laccata. R. luteolus infected seedlings attained minimum growth at this light intensity with 1P level. No such marked differences in the growth of pine seedlings at different levels of phosphorus were observed under low light intensity (Table 4.1).

(ii) Effect of temperature

Colonization of mycorrhizae and growth of pine seedlings were found maximum at 25°C temperature. Slow seedling growth was obtained at 10°C. Colonization of mycorrhizae was also slow at this temperature (Table 4.19). Higher temperature (30°C) was quite unfavourable for the colonization of mycorrhizae and growth of pine seedlings (Table 4.14 and Plate 8). Seedling mortality was highest at 30°C before the first harvest and thereafter, seedlings dried

gradually. At the end of second harvest not a single seedling was found surviving.

1/2P level was found most favourable to promote colonization of mycorrhiza and the growth of seedlings contrasting the results obtained in light intensity experiment (Tables 4.19 and 4.14). Maximum shoot height was observed by seedlings with L. laccata followed by P. tinctorius at 1/2P level at 25°C. Minimum shoot height was obtained by seedlings inoculated with R. luteolus at this temperature with 1/2P level (Table 4.14). Percent colonization of the root was also high at 1/2P level with L. laccata (Fig. 4.19).

Seedlings with P. tinctorius obtained highest percentage of mycorrhizal colonization (Table 4.19) and seedling growth with C. radicata at 10°C with 1/2P level (Table 4.14). It was followed by R. luteolus and L. laccata while, minimum seedling growth was obtained by seedlings with P. tinctorius (Table 4.14). Insignificant difference in colonization of mycorrhiza and seedling growth was observed between 1P and 2P levels of soil phosphorus (Table 4.19).

Maximum shoot and root dry weights were also obtained at 1/2P level under both the temperature levels (25°C and 10°C) but dry weight was more at 25°C temperature compared to 10°C (Tables 4.15-4.18). Root and shoot dry matter production was found affected by the duration of harvest (Table 4.21-4.24) and by different species except at 10°C (Table 4.23).

(iii) Effect of relative humidity (%)

Development of mycorrhiza and growth of seedlings were almost similar at both the levels of humdity. Seedlings with P. tinctorius attained maximum growth compared to others (Table 4.25).

1/2P level of soil phosphorus was found most suitable for the mycorrhizal development and the growth of seedlings at both the levels of humidity. 1P and 2P levels of phosphorus did not exhibit any significant variation both on

the development of mycorrhiza and growth of seedlings (Tables 4.31 and 4.25). Maximum shoot and root dry matter was produced by P. tinctorius. No significant variation in dry matter production was observed by seedlings with other fungi (Table 4.33-4.36) except shoot dry matter at high level of relative humidity (Table 4.34).

B. Survival of seedlings

(i) Effect of light

Survival of the seedlings was higher under moderate light intensity than high light intensity. Lowest rate of seedling survival was obtained under low light intensity (Table 4.9). Different levels of phosphorus did not affect the survival of pine seedlings significantly.

Maximum survival percentage of seedlings was noticed with P. tinctorius which was followed by L. laccata inoculated seedlings. Minimum survival percentage was noticed in uninoculated seedlings under all the light intensities (Table 4.9).

(ii) Effect of temperature

Percentage survival of the seedlings was lowest at 30°C and highest at 25°C. Survivality of the seedlings was affected by the levels of soil phosphorus. At 1/2P level of soil phosphorus survival was found highest and the lowest survival was obtained at 2P level. However, minimum survival percentage was observed in uninoculated seedlings at all the levels of phosphorus. At 25°C minimum survival was noticed with L. laccata with 2P level while survival percentage was lowest with P. tinctorius at 1P level. Similar trend was observed at 10°C with 2P level of soil phosphorus (Table 4.20).

(iii) Effect of relative humidity (%)

Low level of humidity decreased the survivorship of the seedlings

compared to high level (Table 4.32) 1/2P level of the soil phosphorus was found to enhance the survivorship of seedlings in this case also.

Survivorship was noticed minimum with L. laccata and maximum with P. tinctorius at low level of humidity while survivorship was not found significantly varied with high level (Table 4.32).

Discussion

Higher rate of mycorrhizal development at high light intensity may be attributed to the increased amount of photosynthate available to the mycobiont (Bjorkman, 1942). Regulation of soluble sugars in the root tissue of the host plant may be influenced by the light intensity which promoted better mycorrhizal development under high light intensity than at low light regime. The hypothesis on the regulation of carbon energy in heterotrophs due to light has also been supported by other workers (Harley and Waid, 1955; Wenger, 1955; Hacskaylo and Snow, 1959; Boullard, 1961; Shemakhanova, 1962). Studies revealed that active photosynthesis was necessary for the development of mycorrhiza only after their primary leaves have developed (Harley, 1948; Warren-Wilson, 1951; Robertson, 1954; Boullard, 1960, 1961; Laiho and Mikola, 1964).

Considerably less mycorrhizae under moderate and no mycorrhiza under low light intensity has also been attributed to the slow rate of photosynthate to the mycorrhizal fungi which was directly proportional to the amount of soluble sugars in the root tissue (Bjorkman, 1942). Lack of mycorrhiza under low light intensity might be due to unability of the fungal auxins to induce the specific physiological and metabolic changes in the roots which are required for establishment of the symbiotic relationship (Slankis, 1963, 1973). Lack of mycorrhiza at low light intensity can, therefore, be coupled

with deficiency of soluble sugars and auxins required to induce the changes in morphology of roots (Slankis, 1951, 1973; Harley and Lewis, 1969; Kozlowski, 1971). Different levels of phosphorus have also not induced the development of mycorrhiza probably due to limited supply of carbon especially at low irradiation (Stribley and Snellgrove, 1985; Smith et al., 1986). However, better mycorrhizal development and growth of pine seedlings at 1/2P level under moderate light intensity suggested that some amendment of low fertile soil was essential to improve the efficiency of mycorrhizal fungi. Tester etal. (1985) and Smith et al. (1986) have also reported low uptake under low light regimes.

Maximum colonization by mycorrhizal fungi and growth of pine seedlings at 25°C was correlated to the adaptability of the mycobionts (Hacskaylo et al., 1965; Harley, 1969). Bowen (1970) correlated the catalyst of root metabolism and exudation to the development of mycorrhiza at 25°C than 10°C which might have produced insufficient root exudates for the colonization. However, colonization process may also be influenced by different fungal species (Marx et al., 1970).

No significant differences in colonization and growth of pine seedlings was found at the two levels of relative humidity. The possible reasons for this may be either due to a less difference between the high (70% - 100%) and low (50% - 65%) levels of relative humidity or very low irradiance (300 lux approx.) available in the growth chamber.

Application of double dose of phosphorus to the seedlings under high light intensity enhanced the mycorrhizal colonization as well as the seedling growth which has contradicted the findings of Hacskaylo and Snow (1959), who were of the opinion that application of fertilizers above moderate level reduced the mycorrhizal development under high light intensity. However,

exact interpretation of his results may be obtained only after getting his light exposure data. Seedling growth and mycorrhizal colonization was slightly increased at half normal (1/2P) dose of phosphorus than the normal dose of phosphorus under moderate intensity which suggested that better carbon supply to mycobionts at a threshold level of phosphorus may be most suitable for maintaining the phosphorus uptake. Low phosphorus uptake by mycorrhizal seedlings under low light intensity has been reported by some workers (Bhat, 1982; Tester et al., 1985; Smith et al., 1986).

Mycorrhizal fungi enhanced the survival and growth rates of seedlings under different climatic conditions (Fakuara, 1988). In the present study, seedlings with P. tinctorius produced marked growth compared to others. Similar superiority of the mycobiont has also been noticed elsewhere (Valdes, 1985). Marx (1977) supported the findings that certain species of fungal symbionts exert more beneficial effects than others. C. radicata and R. luteolus inoculated seedlings survived better than L. laccata inoculated ones. It may, therefore, be suggested that P. tinctorius, C. radicata and L. laccata can be exploited in reforestation programme, however, few more trials regarding their field performance are required as they may show some variability in inducing growth and nutrient uptake in pine seedlings in different field condition.

Table 4.1 : Effect of different intensities of light on the growth of shoot and root length (cm) of pine seedlings after 180 days

Intensity		*L. laccata*			*C. radicata*			*R. luteolus*			*P. tinctorius*			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
High	SL	18	18	13	15	15	14	13	16	14	14	14	13	13
	RL	53	60	50	43	53	46	45	55	49	43	51	51	44
Moderate	SL	30	37	30	29	36	28	31	38	30	33	41	36	26
	RL	20	20	14	16	17	14	14	18	16	16	21	20	15
Low	SL	6	7	6	8	10	7	7	9	10	6	9	8	6
	RL	5	6	6	7	7	6	6	8	9	6	6	7	5

1P, 2P and 1/2P are the different doses of phosphorus (SSP); SL = Shoot Length; RL = Root Length

Table 4.2 : Effect of high light intensity on the production of fresh and dry weight (mg) of shoots of pine seedlings

Days		*L. laccata*			*C. radicata*			*R. luteolus*			*P. tinctorius*			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	142	263	150	131	123	125	151	156	136	145	260	161	125
	DW	91	88	9	93	81	88	95	46	90	91	68	97	26
60	FW	171	428	217	266	268	140	168	474	152	282	327	208	161
	DW	108	120	70	112	106	88	98	102	105	91	118	104	98
90	FW	554	792	433	283	425	353	295	668	505	656	527	375	308
	DW	112	361	119	80	120	106	77	223	147	141	121	128	108
120	FW	864	905	812	525	791	644	519	879	735	875	788	598	517
	DW	335	363	341	168	315	301	170	355	305	361	317	188	165
150	FW	985	1221	995	816	1091	874	822	1123	915	1020	979	865	816
	DW	411	562	441	338	511	328	312	521	371	490	488	327	314
180	FW	1150	2025	1520	1325	1810	1412	1440	1885	1561	1710	1665	1460	1395
	DW	623	792	613	621	755	576	711	785	662	710	650	721	720

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.3 : Effect of high light intensity on the production of fresh and dry weight (mg) of roots of pine seedlings

Days		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	17	52	18	11	19	12	18	23	19	19	27	20	15
	DW	10	39	9	8	9	8	9	18	9	10	16	13	2
60	FW	23	495	28	24	13	23	24	439	32	32	260	35	19
	DW	12	80	16	18	32	12	13	57	22	20	37	14	10
90	FW	192	612	150	180	195	104	98	543	207	219	378	252	160
	DW	44	123	34	43	45	26	23	111	52	50	101	89	38
120	FW	300	714	311	312	340	214	224	665	367	405	567	379	315
	DW	99	215	112	112	115	99	100	133	134	122	129	125	101
150	FW	417	825	443	411	1534	372	365	871	600	427	782	585	525
	DW	127	235	130	119	131	109	125	231	187	127	212	178	129
180	FW	716	979	665	688	750	623	577	995	871	625	950	868	612
	DW	214	232	212	141	152	142	157	242	214	141	205	188	165

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.4 : Effect of moderate light intensity on the production of fresh and dry weight (mg) of shoots of pine seedlings

Days		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	92	261	125	62	162	101	109	211	141	82	122	102	90
	DW	11	43	12	22	31	92	115	55	95	16	75	101	10
60	FW	126	448	310	112	290	222	224	317	272	142	253	195	167
	DW	23	75	81	52	62	104	101	116	121	33	124	109	28
90	FW	254	562	367	322	405	221	259	467	248	267	392	137	256
	DW	57	120	122	69	110	120	122	231	156	53	130	115	56
120	FW	764	915	822	525	805	652	520	880	755	880	790	612	520
	DW	95	220	351	123	210	310	181	448	392	160	371	238	168
150	FW	995	1320	1012	882	1190	882	835	1223	927	1100	992	870	812
	DW	235	364	461	169	314	342	374	554	396	360	518	389	301
180	FW	1750	2220	1720	1420	1921	1512	1620	1995	1661	1825	1720	1660	1405
	DW	401	562	640	339	512	638	774	825	689	499	689	835	340

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.5 : Effect of moderate light intensity on the production of fresh and dry weight (mg) of roots of pine seedlings

Days		*L. laccata*			*C. radicata*			*R. luteolus*			*P. tinctorius*			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	22	58	12	18	43	15	13	62	21	12	66	11	19
	DW	2	9	2	3	9	1	2	11	10	4	6	4	2
60	FW	54	147	36	43	91	31	41	103	87	26	112	41	43
	DW	4	14	3	9	15	4	5	23	21	8	12	11	5
90	FW	81	192	75	103	143	89	83	163	63	96	182	59	97
	DW	8	21	5	12	20	7	10	36	10	9	23	9	10
120	FW	132	412	152	180	242	104	162	541	113	169	378	152	160
	DW	14	20	9	22	41	12	18	56	19	16	61	13	9
150	FW	300	612	312	301	320	212	221	615	347	492	527	349	312
	DW	44	123	34	41	49	25	24	101	55	51	111	90	41
180	FW	407	799	433	401	522	361	325	821	585	412	722	525	515
	DW	99	225	122	115	125	101	101	132	120	124	141	125	120

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.6: Effect of low light intensity on the production of fresh and dry weight(mg)of shoots of pine seedlings

Days		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	18	22	20	15	21	22	15	18	18	19	17	17	14
	DW	9	9	10	9	11	11	9	9	9	9	9	10	9
60	FW	20	21	24	21	20	23	22	21	20	22	18	25	15
	DW	9	10	10	10	9	10	11	9	9	10	9	10	9
90	FW	22	30	30	22	25	25	21	28	27	28	27	26	22
	DW	10	12	13	11	11	12	10	13	15	14	13	14	11
120	FW	23	31	30	20	25	29	27	31	32	34	37	32	26
	DW	11	13	14	11	12	13	13	14	14	15	16	15	12
150	FW	35	41	51	40	38	41	39	42	44	44	46	39	35
	DW	16	18	21	19	18	17	17	20	21	19	21	20	16
180	FW	56	61	62	52	66	62	70	67	68	71	72	78	49
	DW	20	28	27	22	30	29	28	27	30	31	37	30	21

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.7: Effect of low light intensity on the production of fresh and dry weight(mg)of roots of pine seedlings

Days		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	9	9	8	5	8	8	6	5	6	8	9	6	5
	DW	3	3	3	2	3	3	2	2	2	3	3	2	2
60	FW	9	9	10	8	8	9	5	8	7	10	11	8	4
	DW	4	3	4	2	2	3	1	2	2	4	4	2	1
90	FW	12	14	10	12	15	12	11	17	11	14	12	14	11
	DW	5	4	3	4	4	4	3	5	3	4	3	4	3
120	FW	15	15	11	16	15	10	15	18	18	18	17	15	13
	DW	5	4	3	5	4	5	5	6	6	6	5	5	4
150	FW	17	18	18	18	21	23	24	23	22	32	20	27	15
	DW	8	8	9	9	9	10	8	9	8	9	8	8	8
180	FW	21	22	22	22	22	25	29	25	23	31	31	34	24
	DW	10	9	10	10	9	10	12	12	11	12	12	12	9

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.8 : Effect of different light intensities on colonization (%) of ectomycorrhizae of pine after 180 days by different ectomycorrhizal fungi

Intensity	L. Laccata			C. radicata			R. luteolus			P. tinctorius		
	1P	2P	1/2P	1P	2P	1/P	1P	2P	1/2P	1P	2P	1/2P
High	85	99	80	80	85	82	84	90	81	72	75	73
Moderate	69	80	71	65	75	66	60	70	62	73	85	71
Low	0	0	0	0	0	0	0	0	0	0	0	0

1P, 2P and 1/2P are the different doses of phosphorus (SSP)

Table 4.9 : Survival (%) of the pine seedlings under different intensities of light

Intensity		30 Days			60 Days			90 Days			120 Days			150 Days			180 Days		
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P
High	A	100	100	95	95	95	90	85	90	85	80	85	75	80	80	75	75	75	70
	B	100	100	95	85	90	90	80	85	80	75	80	75	75	80	70	70	75	70
	C	100	100	95	85	95	85	80	85	75	75	85	70	70	75	70	65	70	60
	D	100	100	100	95	100	95	90	95	90	90	90	85	85	90	85	80	85	80
	Co.	90	95	90	80	85	80	80	80	70	65	70	65	60	65	55	50	60	45
Moderate	A	100	100	100	95	100	90	90	90	85	80	80	85	80	80	80	80	80	80
	B	100	100	100	90	90	85	80	85	85	80	80	80	75	80	75	75	75	75
	C	100	100	100	85	90	80	80	85	80	75	85	80	75	80	70	70	75	65
	D	100	100	100	100	100	100	95	100	95	90	100	90	80	95	90	82	90	80
	Co.	100	100	100	90	90	85	80	85	80	70	75	75	60	70	60	50	60	50
Low	A	90	100	90	80	80	80	65	70	60	60	50	60	50	50	55	20	20	10
	B	100	100	100	80	90	80	70	80	60	50	60	45	35	45	30	10	20	10
	C	90	100	100	85	80	90	75	75	80	55	70	60	40	50	40	15	25	15
	D	100	100	90	80	100	80	70	70	60	60	50	50	55	45	40	25	20	20
	Co.	60	65	65	40	40	35	20	20	20	15	15	10	15	10	5	10	10	5

1P, 2P and 1/2P are different doses of phosphorus (SSP)

A = L. Laccata; B = C. radicata; C = R. luteolus; D = P. tinctorius and Co. = Control.

Table 4.10 : Analysis of variance for the dry matter production of pine roots at high light intensity

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	0.25892	5	0.05178	126.41263*	2.71
Effect of species	0.00382	3	0.00127	3.11237	3.39
Effect of P levels	0.00210	2	0.00105	2.56381	3.49
Effect of days x species	0.01430	15	0.00095	2.32771*	2.21
Effect of days x P levels	0.02263	10	0.00226	5.52515*	2.37
Effect of species x P levels	0.00262	6	0.00044	1.06656	2.68
Error	0.02908	30	0.00041	0	-
Total	0.33349	71	0.00470	0	-

* Significant at P = 0.05

Table 4.11 : Analysis of variance for the dry matter production of pine shoots at high light intensity

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	2.06221	5	0.41244	136.56989*	2.71
Effect of species	0.09710	3	0.03237	10.71711*	3.39
Effect of P levels	0.01604	2	0.00802	2.65609	3.49
Effect of days x species	0.21318	15	0.01421	4.70584*	2.21
Effect of days x P levels	0.08873	10	0.00887	2.93816*	2.37
Effect of species x P levels	0.02054	6	0.00342	1.13337	2.68
Error	0.21442	30	0.00302	0	-
Total	2.71222	71	0.03820	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 4.12 : Analysis of variance for the dry matter production of pine roots at moderate light intensity

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	133878.609	5	26775.722	280.210*	2.71
Effect of species	1317.054	3	439.018	4.594*	3.39
Effect of P levels	10562.859	2	8281.430	55.271*	3.49
Effect of days x species	4994.280	15	332.952	3.484*	2.21
Effect of days x P levels	5174.641	10	517.464	5.415*	2.37
Effect of species x P levels	2356.695	6	392.783	4.111*	2.68
Error	6784.470	30	95.556	0	-
Total	165068.609	71	2324.910	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 4.13 : Analysis of variance for the dry matter production of pine shoots at moderate light intensity

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	2797320.417	5	559464.083	471.458*	2.71
Effect of species	156444.667	3	52148.222	43.945*	3.39
Effect of P levels	166945.333	2	83472.667	70.342*	3.49
Effect of days x species	85115.583	15	5674.372	4.782*	2.21
Effect of days x P levels	70674.250	10	7067.425	5.956*	2.37
Effect of species x P levels	39720.333	6	6620.056	5.579*	2.68
Error	84253.417	30	1186.668	0	-
Total	3400474.000	71	47894.000	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 4.14 : Effect of different temperatures on the growth of shoot and root length (cm) of pine seedlings after 90 days

Temp.(°C)		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
25	SL	4	3	5	5	4	6	3	4	5	4	4	5	3
	RL	8	8	14	13	12	11	15	9	9	10	8	14	10
10	SL	4	4	5	5	6	5	5	4	4	5	4	6	4
	RL	14	9	14	10	12	23	11	12	16	13	10	14	17

1P, 2P and 1/2P are the different doses of phosphorus (SSP); SL = Shoot Length; RL = Root Length

Table 4. 15: Effect of temperature (25°C) on the production of fresh and dry weight (mg) of shoots of pine seedlings

Days		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	150	105	165	146	130	160	185	95	142	115	85	160	115
	DW	29	22	43	30	19	47	42	25	22	22	23	24	20
60	FW	255	162	265	246	139	237	250	120	260	198	116	236	210
	DW	49	29	53	47	42	68	50	33	41	36	32	42	53
90	FW	370	273	391	410	296	397	360	238	425	303	218	328	261
	DW	75	33	88	49	44	76	59	40	53	39	52	79	53

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.16 : Effect of temperature (25°C) on the production of fresh and dry weight (mg) of roots of pine seedlings

Days		*L. laccata*			*C. radicata*			*R. luteolus*			*P. tinctorius*			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	40	52	92	93	65	60	65	40	80	86	70	80	10
	DW	2	9	10	3	3	11	9	3	3	3	2	2	1
60	FW	113	61	152	127	75	123	97	63	152	136	81	199	78
	DW	7	10	20	18	7	15	14	7	15	13	8	15	8
90	FW	268	152	194	196	144	183	197	109	255	262	133	258	102
	DW	16	9	26	26	9	30	17	16	22	20	9	18	10

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.17 : Effect of temperature (10°C) on the production of fresh and dry weight (mg) of shoots of pine seedlings

Days		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	145	72	86	149	93	136	84	130	113	109	80	132	74
	DW	25	26	42	26	18	35	25	34	30	28	15	40	35
60	FW	230	152	186	150	196	165	150	136	170	235	180	230	108
	DW	48	24	53	49	97	45	28	43	37	36	26	44	23
90	FW	310	297	341	322	280	375	321	215	397	300	200	301	210
	DW	74	50	54	50	62	54	55	74	53	54	42	65	57

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.18 : Effect of temperature (10°C) on the production of fresh and dry weight (mg) of roots of pine seedlings

Days		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	89	18	95	81	22	77	36	23	81	68	34	72	31
	DW	4	2	9	2	2	21	2	9	9	14	5	14	4
60	FW	115	65	110	99	60	100	60	55	110	103	65	88	71
	DW	16	16	24	12	8	24	34	16	17	18	8	20	25
90	FW	271	190	232	135	107	180	193	95	211	188	96	126	111
	DW	35	18	30	17	18	33	28	25	35	20	22	26	24

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.19 : Effect of different temperatures on colonization (%) of ectomycorrhizae of pine after 90 days by different ectomycorrhizal fungi

Temp.(°C)	L. Laccata			C. radicata			R. luteolus			P. tinctorius		
	1P	2P	1/2P	1P	2P	1/P	1P	2P	1/2P	1P	2P	1/2P
25°C	55	31	62	42	32	45	38	30	32	48	41	53
10°C	41	29	50	42	24	44	28	22	30	43	30	51

1P, 2P and 1/2P are the different doses of phosphorus (SSP)

Table 4.20 : Survival (%) of the pine seedlings at different temperatures

Temp. (°C)		30 Days			60 Days			90 Days		
		1P	2P	1/2P	1P	2P	1/P	1P	2P	1/2P
10	A	80	30	90	67	50	100	100	80	100
	B	90	67	100	70	33	100	100	67	100
	C	70	60	100	70	67	100	90	80	100
	D	90	90	100	100	100	100	100	100	100
	Co.	60	50	60	50	60	60	60	60	60
25	A	100	67	100	50	33	67	100	67	100
	B	67	67	100	100	100	100	100	67	100
	C	100	67	100	67	100	100	100	100	100
	D	100	67	100	33	33	100	67	100	100
	Co.	60	100	60	33	33	33	33	50	33
30	A	33	33	33	0	0	0	Observation not done		
	B	33	33	33	0	0	0			
	C	33	33	67	0	0	0			
	D	33	33	67	0	0	0			
	Co.	50	33	50	0	0	0			

1P, 2P and 1/2P are the different doses of phosphorus (SSP)

A = L. laccata; B = C. radicata; C = R. luteolus; D = P. tinctorius and Co. = Control

Table 4.21 : Analysis of variance for the dry matter production of pine roots at 25°C temperature

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	1945.722	2	522.861	145.753*	4.87
Effect of species	57	3	19.213	5.356*	3.86
Effect of P levels	380.056	2	190.028	52.972*	4.87
Effect of days x species	40.278	6	6.713	1.871	2.51
Effect of days x P levels	137.778	4	34.444	9.602*	2.87
Effect of species x P levels	167.278	6	27.880	7.772*	2.51
Error	125.556	12	3.587	0	-
Total	1954.306	35	35.837	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 4.22 : Analysis of variance for the dry matter production of pine shoots at 25°C temperature

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	4792.388	2	2396.194	154.593*	4.87
Effect of species	442.082	3	147.361	9.507*	3.86
Effect of P levels	2527.055	2	1263.527	81.518*	4.87
Effect of days x species	377.834	6	62.972	4.063*	2.51
Effect of days x P levels	466.612	4	116.653	7.526*	2.87
Effect of species x P levels	1551.834	6	258.638	16.686*	2.51
Error	542.499	12	15.500	0	-
Total	10700.305	35	305.723	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 4.23 : Analysis of variance for the dry matter production of pine roots at 10°C temperature

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	2056.222	2	1028.111	116.578*	4.87
Effect of species	72.750	3	24.250	2.750	3.86
Effect of P levels	495.389	2	247.694	28.086*	4.87
Effect of days x species	174.000	6	29.000	3.288*	2.51
Effect of days x P levels	94.444	4	23.611	2.677	2.87
Effect of species x P levels	298.833	6	49.806	5.647*	2.51
Error	508.667	12	8.817	0	-
Total	3500.306	35	100.009	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 4.24 : Analysis of variance for the dry matter production of pine shoots at 10°C temperature

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	4334.389	2	2167.195	45.637*	4.87
Effect of species	741.639	3	247.213	5.206*	3.86
Effect of P levels	376.223	2	188.111	3.961	4.87
Effect of days x species	1639.611	6	273.268	5.755*	2.51
Effect of days x P levels	252.611	4	63.153	1.330	2.87
Effect of species x P levels	1264.444	6	210.741	4.438*	2.51
Error	1662.056	12	47.487	0	-
Total	102.973	35	293.456	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 4.25 : Effect of different levels of humidity on the growth of shoot and root length (cm) of pine seedlings after 90 days

RH (%)		*L. laccata*			*C. radicata*			*R. luteolus*			*P. tinctorius*			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
High	SL	4	3	5	6	4	5	3	4	6	5	5	6	6
	RL	9	8	11	12	11	12	15	8	12	10	17	15	13
Low	SL	5	5	5	4	5	5	6	4	5	5	6	5	4
	RL	10	11	15	8	9	17	12	10	11	11	8	13	8

1P, 2P and 1/2P are the different doses of phosphorus (SSP); SL = Shoot Length; RL = Root Length
RH = Relative Humidity

Table 4.26 : Effect of high relative humidity (%) on the production of fresh and dry weight (mg) of shoots of pine seedlings

Days		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	145	95	160	146	131	151	175	98	140	105	75	150	120
	DW	28	22	42	28	20	45	41	26	21	20	24	24	21
60	FW	235	152	235	244	140	241	248	120	254	201	126	234	205
	DW	50	30	51	42	42	67	51	34	40	40	35	41	42
90	FW	310	289	335	312	370	365	309	225	394	310	192	300	201
	DW	73	49	50	51	61	52	53	68	53	55	41	66	52

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.27 : Effect of high relative humidity (%) on the production of fresh and dry weight (mg) of roots of pine seedlings

Days		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	42	52	91	90	66	61	65	42	78	79	65	77	12
	DW	2	9	10	3	3	11	9	3	3	3	2	2	1
60	FW	115	62	150	130	71	120	92	60	147	135	82	182	75
	DW	7	10	20	18	7	15	14	7	15	13	8	15	8
90	FW	270	180	233	140	112	175	188	92	202	172	89	119	102
	DW	35	18	30	17	18	33	28	25	35	20	22	26	24

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.28 : Effect of low relative humidity (%) on the production of fresh and dry weight (mg) of shoots of pine seedlings

Days		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	145	72	86	140	95	132	85	131	112	110	83	131	75
	DW	25	26	42	26	18	35	25	34	30	28	15	35	40
60	FW	225	150	185	155	192	165	150	131	168	233	175	231	110
	DW	48	24	53	49	97	45	28	43	37	36	26	44	23
90	FW	370	265	390	411	295	399	350	241	420	300	220	319	252
	DW	75	33	88	49	44	76	59	40	53	39	52	79	40

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.29 : Effect of low relative humidity (%) on the production of fresh and dry weight (mg) of roots of pine seedlings

Days		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
30	FW	88	20	91	80	25	67	35	22	82	64	34	71	29
	DW	4	2	9	2	2	21	2	8	8	13	5	14	4
60	FW	110	68	109	95	62	102	63	51	101	112	68	87	70
	DW	16	16	24	12	12	22	32	16	17	18	8	20	25
90	FW	260	150	195	188	151	178	201	110	249	265	135	260	100
	DW	16	9	26	26	9	30	17	16	22	20	9	18	10

1P, 2P and 1/2P are the different doses of phosphorus (SSP); FW = Fresh Weight; DW = Dry Weight

Table 4.31 : Effect of different levels of humidity on colonization (%) of ectomycorrhizae of pine after 90 days by different ectomycorrhizal fungi

RH (%)	L. Laccata			C. radicata			R. luteolus			P. tinctorius		
	1P	2P	1/2P	1P	2P	1/P	1P	2P	1/2P	1P	2P	1/2P
High	45	31	50	38	28	45	35	25	40	51	32	55
Low	38	24	43	32	27	39	29	22	32	49	28	52

1P, 2P and 1/2P are the different doses of phosphorus (SSP)

Table 4.32 : Survival (%) of the pine seedlings at different humidity levels

RH (%)		30 Days			60 Days			90 Days		
		1P	2P	1/2P	1P	2P	1/P	1P	2P	1/2P
	A	75	55	90	90	60	90	90	70	100
	B	85	67	100	100	70	100	100	80	100
High	C	85	70	90	95	67	95	95	80	100
	D	100	80	100	100	70	100	100	70	100
	Co.	50	50	55	60	60	65	60	70	65
	A	80	75	80	75	55	80	80	50	80
	B	80	45	80	80	60	90	85	60	85
Low	C	70	60	90	80	50	75	85	60	90
	D	80	60	100	80	60	100	90	60	90
	Co.	50	40	50	40	40	50	50	50	50

RH = Relative Humidity; 1P, 2P and 1/2P are the different doses of phosphorus (SSP)

A = L. laccata; B = C. radicata; C = R. luteolus; D = P. tinctorius and

Co. = Control

Table 4.33 : Analysis of variance for the dry matter production of pine roots at high level of relative humidity

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	2608.167	2	1304.083	157.088*	4.87
Effect of species	64.889	3	21.630	2.605	3.86
Effect of P levels	288.167	2	144.083	17.356*	4.87
Effect of days x species	79.611	6	13.269	1.598	2.51
Effect of days x P levels	75.667	4	18.917	2.279	2.87
Effect of species x P levels	56.944	6	9.491	1.143	2.51
Error	290.556	12	8.302	0	-
Total	3464.000	35	98.971	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 4.34 : Analysis of variance for the dry matter production of pine shoots at high level of relative humidity

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	5675.724	2	2837.862	90.694*	4.87
Effect of species	510.085	3	170.028	5.434*	3.86
Effect of P levels	322.724	2	161.362	5.157*	4.87
Effect of days x species	427.832	6	71.305	2.279	2.51
Effect of days x P levels	777.943	4	194.486	6.215*	2.87
Effect of species x P levels	604.165	6	100.694	3.218*	2.51
Error	1095.168	12	31.291	0	-
Total	9413.641	35	268.961	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 4.35 : Analysis of variance for the dry matter production of pine roots at low level of relative humidity

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	862.167	2	431.083	60.770*	4.87
Effect of species	26.778	3	8.926	1.258	3.86
Effect of P levels	582.167	2	291.083	41.034*	4.87
Effect of days x species	196.722	6	32.787	4.622*	2.51
Effect of days x P levels	128.167	4	32.042	4.517*	2.87
Effect of species x P levels	264.722	6	44.120	6.220*	2.51
Error	248.278	12	7.094	0	-
Total	2309.000	35	65.971	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 4.36 : Analysis of variance for the dry matter production of pine shoots at low level of relative humidity

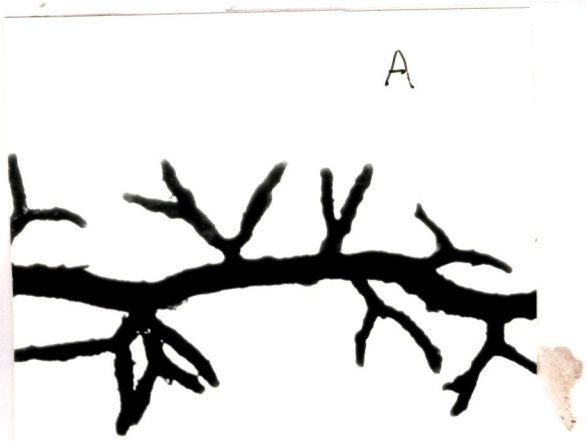
Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of days	4910.724	2	2455.362	37.723*	4.87
Effect of species	638.530	3	212.843	3.270	3.86
Effect of P levels	366.057	2	683.029	10.494*	4.87
Effect of days x species	1359.720	6	226.620	3.482*	2.51
Effect of days x P levels	1170.109	4	292.527	4.494*	2.87
Effect of species x P levels	1604.387	6	267.398	4.108*	2.51
Error	2278.113	12	65.089	0	-
Total	13327.641	35	380.790	0	-

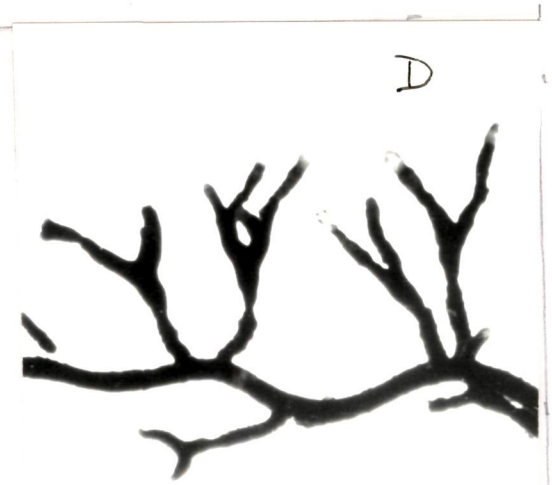
* Significant at P = 0.05

P level = Phosphorus level

Plate - 4 : Morphology of ectomycorrhizae formed by A - L. laccata, B - C. radicata (white arrow indicates the fungal mycelia around the ectomycorrhizae), C - R. luteolus and D - P. tinctorius.

PLATE – 4.





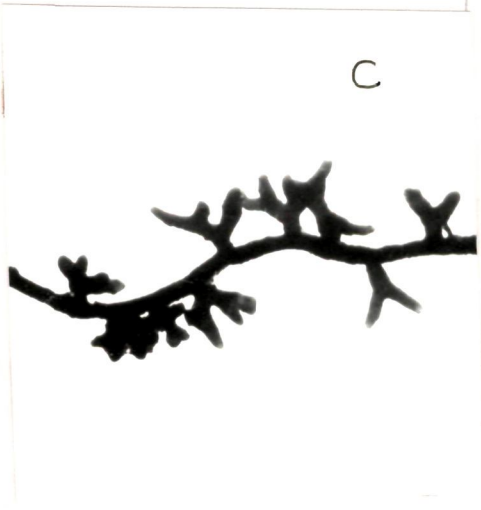
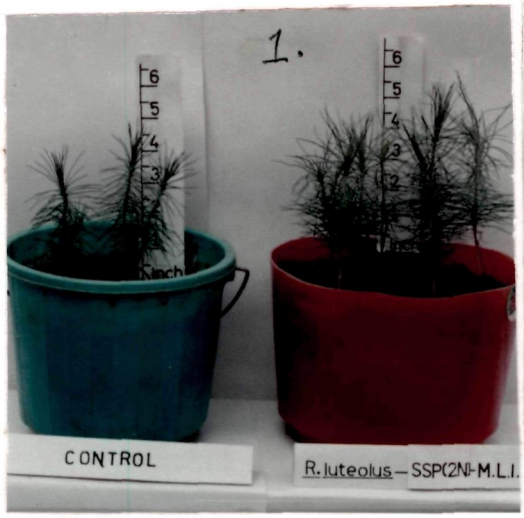
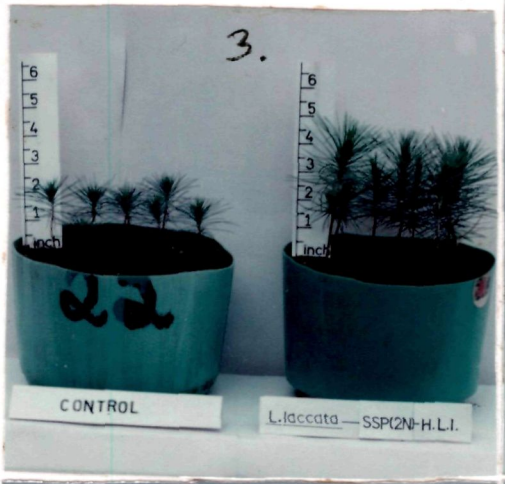


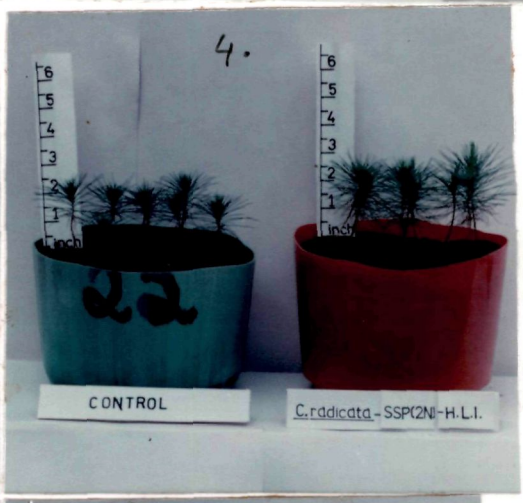
Plate - 5 : Effect of light intensities on the growth of pine (P. kesiya) shoots. Growth of shoots under moderate light intensity infected by R. luteolus (Fig. 1) and P. tinctorius (Fig. 2). Growth of pine shoots under high light intensity infected by L. laccata (Fig. 3), C. radicata (Fig. 4), R. luteolus (Fig. 5) and P. tinctorius (Fig. 6). MLI and HLI are the abbreviations for Moderate and High Light Intensities respectively. SSP (2N) is the 2P level of phosphorus (SSP).

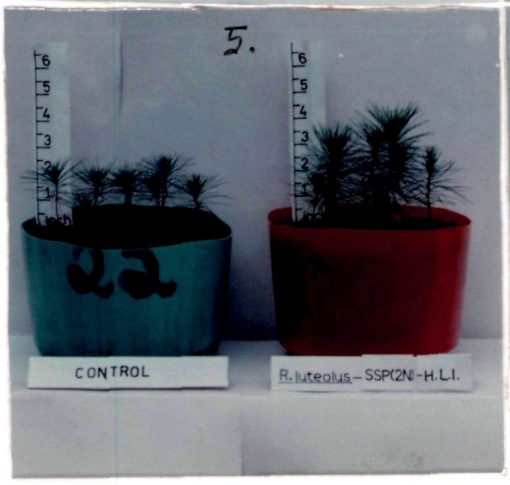
PLATE-5


1.
CONTROL
R. luteolus – SSP(2N)-M.L.I.

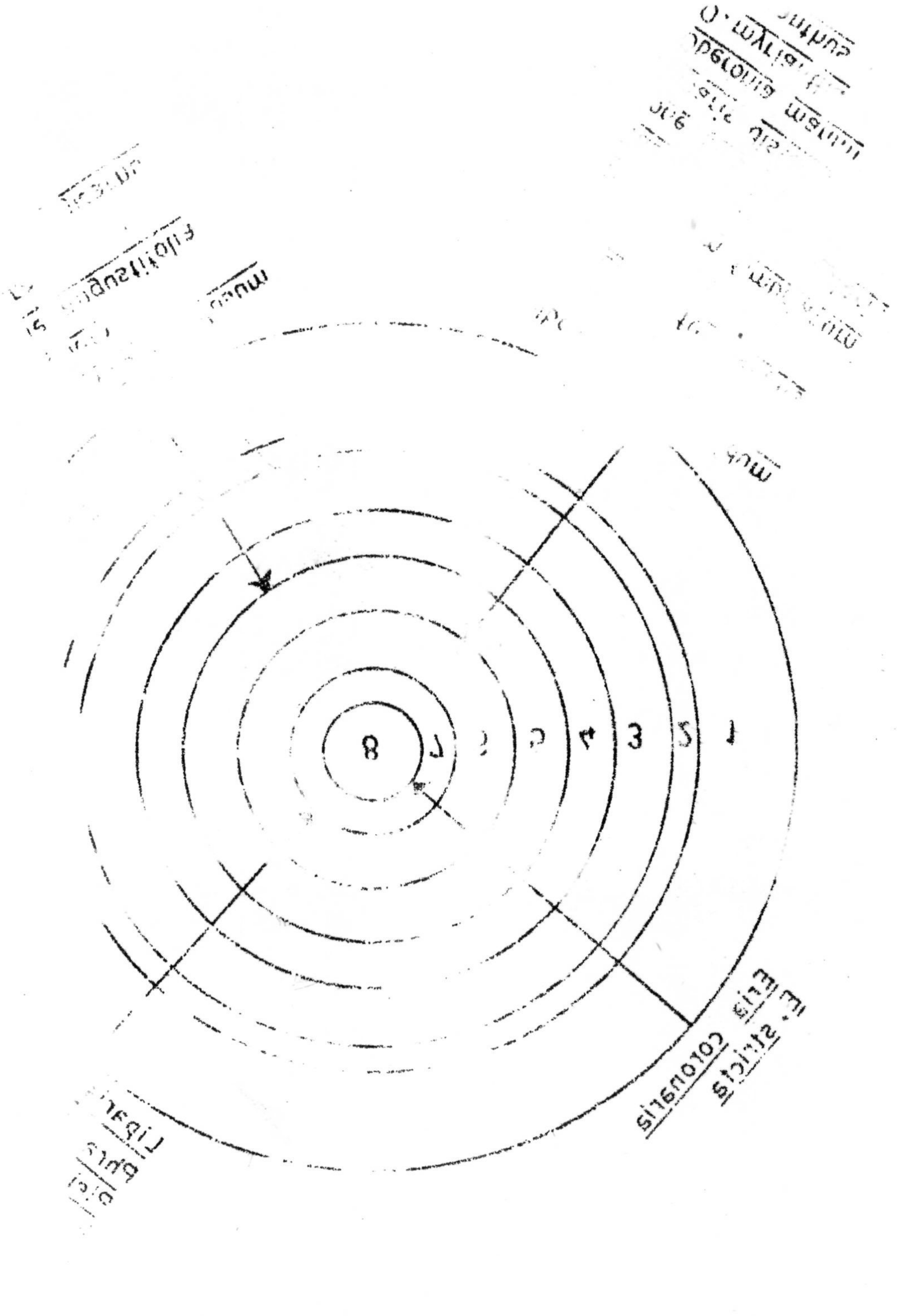

2.
CONTROL
P. tinctorius - SSP(2N)-M.L.I.


3.
CONTROL
L. laccata – SSP(2N)-H.L.I.


4.
CONTROL
C. radicata - SSP(2N)-H.L.I.


5.
CONTROL
R. luteolus – SSP(2N)-H.L.I.


6.
CONTROL
P. tinctorius - SSP(2N)-H.L.I.



Erica coronaris
E. stricta
1
2
3
4
8

Plate - 6 : Comparative study on the effect of different light intensities on shoot growth of pine seedlings infected by L. laccata (Fig. 1), C. radicata (Fig. 2), R. luteolus (Fig. 3) and P. tinctorius (Fig. 4). HLI, MLI and LLI are used for High, Moderate and Low Light Intensities respectively. SSP (2N) is the 2P level of phosphorus (SSP).


1.
-H.L.I.
L.laccata — SSP(2N)-M.L.I.
-L.L.I.
2.
-H.L.I.
C.rubicata — SSP(2N)-M.L.I.
-L.L.I.
3.
-H.L.I.
R.luteolus — SSP(2N)-M.L.I.
-L.L.I.
4.
-H.L.I.
B.tinctorius - SSP(2N)-M.L.I.
-L.L.I.

PLATE - 6

Plate - 7 : Comparative study on the effect of different light intensities on shoot growth of pine seedlings infected by L. laccata (Figs. 1 and 2) and R. luteolus (Fig. 3 and 4). HLI, MLI and LLI are used for High, Moderate and Low Light Intensities respectively. SSP(N) and SSP(N/2) are the 1P and 1/2P levels of phosphorus (SSP).


1.
-H.L.I.
L.laccata – SSP(N) -M.L.I.
-L.L.I.


2.
-H.L.I.
L.laccata – SSP(N2)-M.L.I.
-L.L.I.


3.
-H.L.I.
R.luteolus – SSP(N) -M.L.I.
-L.L.I.

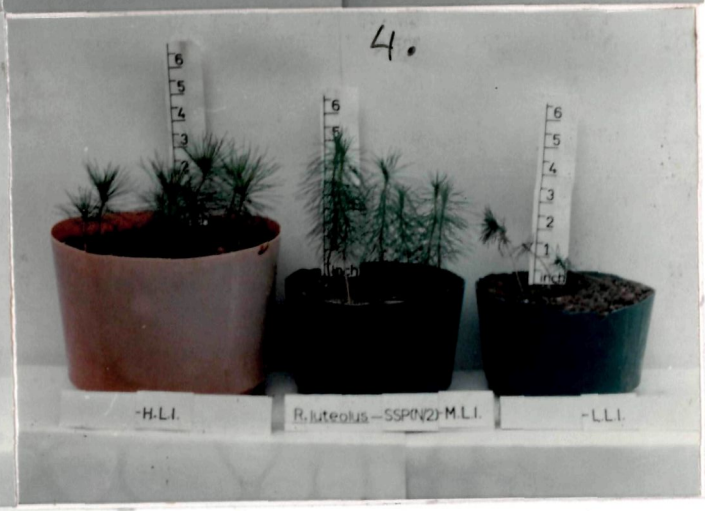
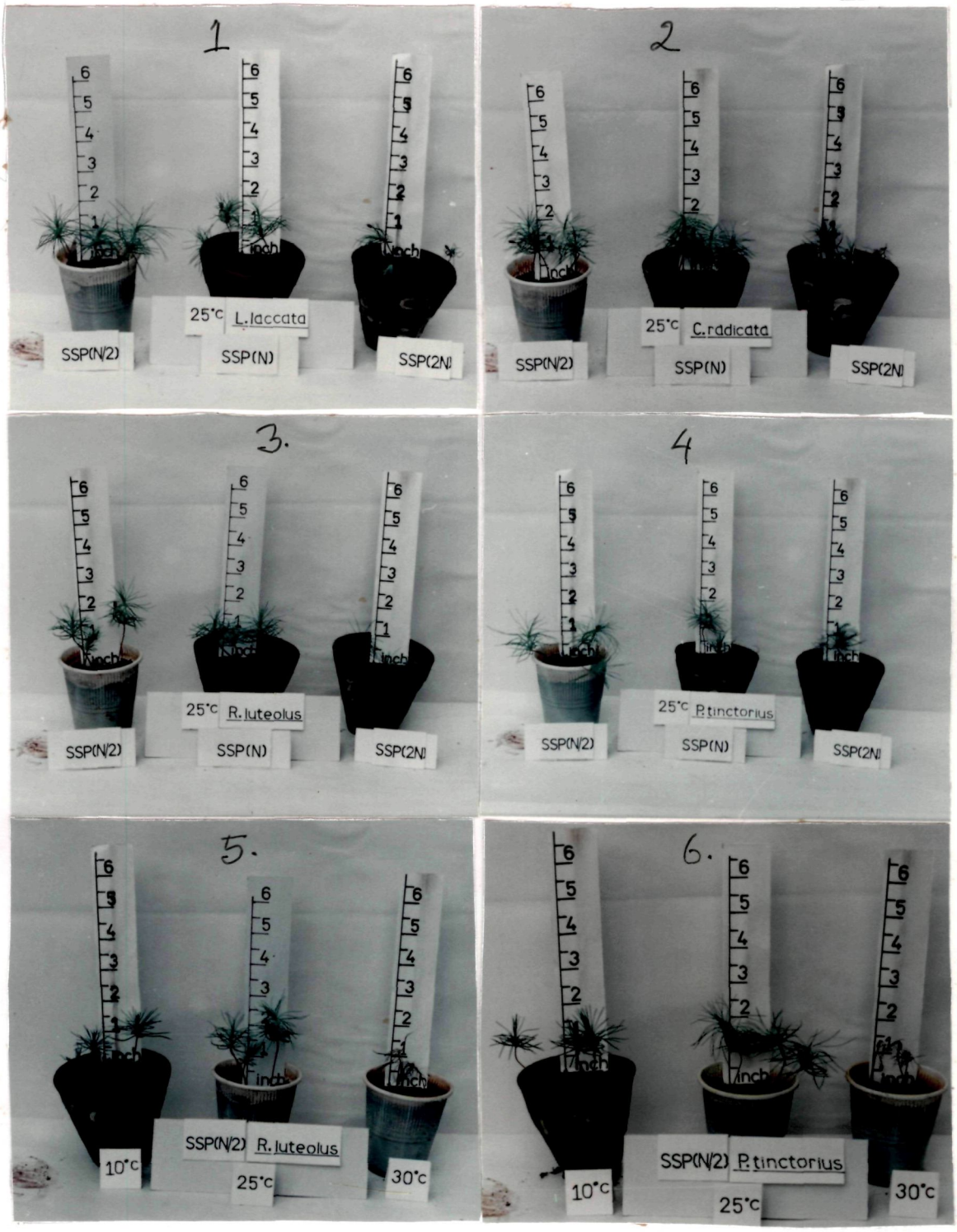

4.
-H.L.I.
R.luteolus – SSP(N2)-M.L.I.
-L.L.I.

PLATE – 7

Plate - 8 : Comparative study on the effect of different doses of phosphorus on the growth of pine shoots infected by L. L. laccata (Fig. 1), C. radicata (Fig. 2), R. luteolus (Fig. 3) and P. tinctorius (Fig. 4) at 25°C. Figs. 5 and 6 show the comparative study on the effect of different temperatures (10°, 25° and 30°C) on the shoot growth of pine seedlings infected by R. luteolus and P. tinctorius respectively. SSP(N/2), SSP(N) and SSP (2N) are used for 1/2P, 1P and 2P levels of phosphorus (SSP).

PLATE-8


1
25°c L.laccata
SSP(N/2)
SSP(N)
SSP(2N)
2
25°c C.radicata
SSP(N/2)
SSP(N)
SSP(2N)
3.
25°c R.luteolus
SSP(N/2)
SSP(N)
SSP(2N)
4
25°c P.tinctorius
SSP(N/2)
SSP(N)
SSP(2N)
5.
SSP(N/2) R.luteolus
10°c
25°c
30°c
6.
SSP(N/2) P.tinctorius
10°c
25°c
30°c

CHAPTER - V

NUTRIENT STATUS (N AND P) OF PINE SEEDLINGS AND PHOSPHATASE ACTIVITY OF ECTOMYCORRHIZAL ROOTS OF PINE UNDER DIFFERENT CLIMATIC CONDITIONS

Introduction

One of the most commonly held hypothesis concerning the effect of mycorrhizas upon nutrient uptake and growth has been that they facilitate the absorption of nitrogen and phosphorus compounds from the soil (Harley, 1969). Although, there have been several reviews in recent years on the enhanced absorption of nitrogen (Raven et al., 1978; Bowen and Smith, 1981; Sharma, 1981; Alexander, 1982) and phosphorus (Theodorou and Bowen, 1970; Harley and McCready, 1981; Sharma, 1981) by the ectomycorrhizal plants. However, informations on the effect of climatic conditions on the uptake of nitrogen and phosphorus are meagre. Son and Smith (1988) have the opinion that uptake of nutrients are influenced by the interactions between ectomycorrhizal symbionts and the environment. Addition of fertilizers above moderate level at high light intensity may reduce the rate of P uptake by mycorrhiza (Hacskaylo and Snow, 1959). Phosphorus uptake may also be reduced at low light intensity (Hayman, 1974; Smith et al., 1986; Son and Smith, 1988).

Temperature is another factor that influenced the absorption of nutrients (Bowen, 1970). Although, informations about the suitable temperature for better

absorption of nutrients are not enough, however, few studies indicate their importance in the nutrient uptake by the plants (Son and Singh, 1988).

Root surface phosphatase activity of the ectomycorrhizal roots may provide some clues to understand the interaction between the climatic factors and mycorrhiza to assess the nutrient uptake. Ridge and Rovira (1971) indicated that root surface phosphatase may be more important in the organic phosphorus mobilization. Several workers (Bartlett and Lewis, 1973; Gianinazzi-Pearson and Gianinazzi, 1976; Alexander and Harley, 1981; Antibus et al., 1981; Dighton, 1983; Dodd et al., 1987) tried to relate the root surface phosphatase activity to the phosphorus mobilization but none of them correated this relationship with the climatic factors. Although, some fragmentary informations in the nutrient uptake under different light intensities are available, but a detailed study is lacking. Therefore, it was planned to study the effect of climatic factors like light, temperature and relative humdity on root surface phosphatase activity and the nutrient uptake by the ectomycorrhizal pine seedlings.

Materials and Methods

Details about the raising of seedlings, transplanting them into the experimental pots, inoculating them by four ectomycorrhizal fungi, setting them at diffeent light intensities, temperatures and humidity levels and harvesting of pine seedlings are described in chapter IV of this thesis. Garden soil with soil properties as follows: pH, 5.5, Organic carbon, 2.27(%), total nitrogen, 0.12 (%) and available phosphorus, 3-7 (ppm) was mixed with the sand in 1:1 ratio and sterilized in the autoclave. Root surface phosphatase activity was measured immediately after harvesting the seedlings. The dry matter of the root and shoot was determined by drying them at 60°C in a hot air oven for 48 hrs. The dried shoot and root tissue were powdered and sieved through .4 mm mesh and nutrient analysis was done according to the following methods and relative absorp-

tion of nutrients was determined comparing the infected seedlings with controlled ones.

Determination of total nitrogen :

Micro-Kjeldahl distillation procedure (outlined by Misra, 1968) was followed to determine the total nitrogen in plant tissue. 0.25 g powdered and sieved plant tissue through .4 mm mesh was rapped in a qualitative Whatman filter paper and dropped in 100 ml Kjeldahl digestion flask. Five ml of H_2SO_4 (Conc.) and .1 g of catalyst mixture (mixture of copper sulphate, potassium sulphate and selenium dioxide in 1:8:1 ratio) were added and kept for digestion for 1 to 1.30 hour. On completion of digestion Kjeldahl flask was left for cooling for 15-20 minutres and thereafter 20 ml of distilled water (NH_4 free) was added. Again the flask was cooled and the contents were transferred into 50 ml volumetric flask and the volume was made up by adding distilled water.

Distilled water was boiled in the flask of distillation unit letting the inlet and outlet be closed and open respectively. When steam passed through the steam jacket 10 ml of aliquot was pipetted out and poured into the digestion chamber with the help of glass funnel. The glass funnel was washed twice wtih 1 ml distilled water. Now ground glass stopper was replaced and 5 ml of 40 per cent NaOH was added. Lower end of the condenser was kept dipped into 5 ml of 2 per cent boric acid in a 50 ml conical flask. When steam issued freely through the tube outlet was clipped and NaOH was allowed to run into the digest and stopper was replaced immediately. Distillation was continued for 30 minutes and the distillate was collected. Four drops of mixed indicator (6 ml methyle red solution, 0.16% in 95% alcohol + 12 ml brown-cresol green, 0.04% in water + 6 ml 95% alcohol) was added to about 50 ml distillate and titrated with $\frac{N}{14}$ HCl. Percent of total nitrogen was calculated from the following formula

$$N(\%) = (T-B) \times 10\ N \times \frac{1.4}{S}$$

where T = Sample titration, ml standard acid

B = Blank titration, ml standard acid

N = Normality of HCl and

S = Weight of plant material (g).

The difference T-B was multiplied by 10 because only 10 ml out of 100 ml digest was distilled.

Determination of Phosphorus :

Phosphorus in plant tissue was determined by molybdenum blue method outlined by Misra (1968). 0.2g dried and sieved (through 0.05 mm mesh) plant matter was ashed by mixing 0.1N Mg $(NO_3)_2$ in a silica basin. The mixture was ignited at 500°C for 30 minutes and dissolved the residue in 10 ml 10N H_2SO_4 and warmed for 15-20 minutes. After that 20 ml distilled water was added and filtered through Whatman No.1 filter paper into a 100 ml volumetric flask. Filter paper was washed several times and the volume was made up.

Ten ml of aliquot of ash solution was pipetted out and transferred to 50 ml volumetric flask. 2 ml of 10N H_2SO_4 was added and volume was made 45 ml by addition of distilled water. One ml ammonium molybdate solution was added and shaken. Then 1 ml of stannous chloride solution was added and shaken immediately. The volume was diluted and after 10 minutes percentage transmission was calculated in colorimeter at 660 nm. Percentage of phosphorus was calculated from the formula :

Phosphorus (%) = Amount of phosphorus in the aliquot (g) x aliquot factor x 100/wt. of sample (g) and converted into ppm.

Assay of phosphatase activity :

Phosphatase activity of the intact mycorrhizal roots was measured by determining the amount of P-nitrophenol released when 1 ml of 50 mM PNP and 4 ml of 0.1M sodium acetate buffer, pH-5.2 was incubated with 100 mg of fresh root tissue as outlined by Dodd et al. (1987). The p-nitrophenol product was developed with NaoH and determined spectro-photometrically at 400 nm.

Results

Nutrient status (Nitrogen and Phosphorus) of pine seedlings :

(i) Effect of light intensities :

Light intensities affected greatly the uptake of nitrogen and phosphorus. It was favoured by high and moderate light intensities but suppressed by low light intensity (Tables 5.1 and 5.2).

More nitrogen concentration was found in the shoots of pine seedlings infected with different ectomycorrhizal fungi under high light intensity compared to modern light intensity. Little less concentration of nitrogen was observed in control set. No variation in nitrogen content in pine seedlings infected with different mycorrhizal fungi was obtained (Table 5.2).

Phosphorus was found readily absorbed by infected seedlings than the controlled one. Phosphorus content was found more in the shoots compared to roots under high light intensity. Reverse trend was observed at moderate light intensity (Table 5.1). Different levels of phosphorus did not affect their absorption at high light intensity except in the case of P. tinctorius infected seedling where highest concentration of root phosphorus was obtained in 2P treated set, but at moderate light intensity root phosphorus of all the infected seedlings was high in 2P treated set. P. tinctorius and L. laccata infected seedlings had the

maximum P content in their roots at 2P level under moderate light intensity. Root and shoot phosphorus was minimum under low light intensity.

Variation in phosphorus content in shoots and roots was found significant at different intensities of light and P levels (Table 5.7 and 5.8) but variation in phosphorus content due to different species of ectomycorrhizal fungi was obtained in roots only (Table 5.7).

(ii) Effect of temperature :

Effect of temperature on the absorption of nitrogen and phosphorus was not significant among different sets at any one temperature but it was considerably high at 25°C than 10°C (Tables 5.3 and 5.4).

Concentrations of nitrogen and phosphorus at 25°C was little more than their control set but no such difference in concentration of nitrogen and phosphorus was observed at 10°C (Table 5.3 and 5.4).

(iii) Effect of humidity :

At both the humidity levels little difference in concentrations of phosphorus and nitrogen content was observed between infected seedlings and control seedlings. Though there was little variation in concentrations in the seedlings infected with different mycobionts, but it was not significant (Table 5.5 and 5.6).

Effect of two levels of humidity was not found affecting the uptake of nitrogen and phosphorus.

Phosphatase activity of the pine roots :

Acid and alkaline phosphatase activities was high under high light and moderate light intensities and low under low light intensity.

Alkaline phosphatase activity was reduced than the acid phosphatase acti-

vity. No significant difference either between control and infected or among the infected roots were observed (Table 5.9).

Alkaline phosphatase activity was quite low than acid phosphatase activity at 25°C but no such difference was found at 10°C (Table 5.10). Enhanced activity of acid phosphatase was noticed at 25°C compared to 10°C but alkaline phosphatase activity was more at 10°C (Table 5.10). Low level of humidity was found more suitable to produce acid phosphatase activity of roots compared to high level. Variation in alkaline phosphatase activity was not observed at these two levels (Table 5.11).

Discussion

Increased absorption of phosphorus and nitrogen by the mycorrhizal plants than non-mycorrhizal ones under high and moderate light intensities is in conformity with the view of Bjorkmann (1942) who advocated that higher light intensity increased the quantity of sugar in tissue and so encouraged the growth of the fungi when the intensity of infection was also increased. Increased intensity of infection suggested the increased uptake of nitrogen and phosphorus as Hatch (1937) had reported that internal nutrient status was the prime factor in determining the intensity of infection.

Maximum uptake of phosphorus at 2P level under high as well as moderate light intensity contradicted the findings of Bjorkman (1942), who explained that under high light intensity addition of phosphate decreased the mycorrhizal infection, and Son and Smith (1988), who showed positive mycorrhizal response at high irradiance without additional phosphorus. The present finding suggests that 2P level was quite low than that of Bjokman's toxic level.

Phosphorus and nitrogen contents in the seedlings were not significantly increased than controls under low light intensity which could be correlated to

the Hayman's (1974) view who suggested reduced phosphorus uptake at low irradiance (Bhat, 1982; Tester et al., 1985; Smith et al., 1986).

Slight increased amount of phosphorus and nitrogen at 25°C was observed that strengthened the view of Harley (1969) who observed slow uptake of nitrogen and phosphorus at 0°C which subsequently increased upto 15°C to 20°C. Uptake was not influenced at 10°C by the symbiotic association which again is in agreement with the above view.

Increased rate of uptake at two levels of humidity could be attributed to the Harley's (1969) view, who found increased rate upto 15° to 20°C as both the levels of humidity were set at a constant temperature (25°C) in the present study.

Higher rate of acid phosphatase activity in different mycorrhizal fungi under different light intensities could be attributed to their capacity to hydrolyse phosphate ions better and more efficiently at an optimum temperature.

The present study suggests the use of 2P level of phosphorus for better growth of seedlings and use of P. tinctorius, L. laccata and R. luteolus under high and moderate light intensities for better growth of the seedlings at 25°C.

Table 5.1 : Phosphorus contents (ppm) in the root and shoot tissues of pine seedlings under different light intensities

Intensity		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
High	Root	6.1	5.3	4.3	5.3	6.2	5.1	8.1	7.8	6.1	4.4	14.9	6.1	8.8
	Shoot	8.8	10.7	4.7	6.6	9.3	6.1	7.5	5.9	5.5	5.2	5.7	7.1	3.4
Moderate	Root	10.1	12.4	9.8	6.7	8.8	5.2	6.4	11.2	8.1	6.7	12.5	8.5	9.1
	Shoot	7.6	5.3	6.8	4.8	6.6	6.1	5.2	6.4	4.6	7.1	10.1	7.2	4.2
Low	Root	5.2	7.1	3.3	4.3	5.2	3.7	5.1	10.4	4.2	5.5	11.2	6.2	8.1
	Shoot	3.1	4.1	3.4	3.1	3.2	3.1	3.4	4.4	3.7	2.1	3.2	2.3	3.2

1P, 2P and 1/2P are different doses of phosphorus (SSP)

Table 5.2 : Nitrogen contents (%) in the root and shoot tissues of pine seedlings under different light intensities

Intensity		*L. laccata*			*C. radicata*			*R. luteolus*			*P. tinctorius*			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
High	Root	1.4	1.3	1.3	1.2	1.3	1.2	1.12	1.2	1.1	1.6	1.7	1.2	1.0
	Shoot	1.5	1.4	1.6	1.1	1.0	1.0	1.1	0.90	0.88	1.6	2.0	1.6	0.64
Moderate	Root	0.9	1.1	1.1	1.08	1.0	0.70	0.91	1.1	1.0	0.91	0.85	1.0	1.1
	Shoot	0.98	1.2	1.3	1.0	0.78	0.71	1.0	0.9	0.8	1.2	1.3	1.1	0.5
Low	Root	0.6	0.5	0.5	0.50	0.34	0.40	0.3	0.23	0.32	0.5	0.6	0.6	0.4
	Shoot	0.5	0.6	0.4	0.3	0.28	0.22	0.3	0.4	0.27	0.6	0.4	0.5	0.4

1P, 2P and 1/2P are different doses of phosphorus (SSP)

Table 5.3 : Phosphorus contents (ppm) in the root and shoot tissues of pine seedlings under different temperatures

Temp. (°C)		*L. laccata*			*C. radicata*			*R. luteolus*			*P. tinctorius*			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
10	Root	5.4	3.2	7.4	4.4	3.5	5.5	5.4	4.2	9.5	6.6	4.5	10.3	2.6
	Shoot	3.8	3.4	4.5	3.2	3.1	3.4	3.6	4.1	5.6	3.8	3.2	6.3	3.2
25	Root	5.9	3.4	8.8	5.1	3.6	6.2	5.6	3.4	9.2	8.5	4.7	12.2	4.2
	Shoot	5.8	3.5	7.6	4.2	3.8	6.1	4.2	4.1	8.6	9.2	3.8	11.1	6.5

1P, 2P and 1/2P are different doses of phosphorus (SSP)

Table 5.4 : Nitrogen contents (%) in the root and shoot tissues of pine seedlings under different temperatures

Temp. (°C)		*L. laccata*			*C. radicata*			*R. luteolus*			*P. tinctorius*			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
10	Root	0.90	0.85	0.80	0.70	0.75	0.90	0.82	0.95	0.65	0.80	0.71	0.68	0.40
	Shoot	0.70	0.80	0.75	0.80	0.65	0.90	0.90	1.05	0.60	0.95	1.10	0.60	0.50
25	Root	0.95	0.90	0.60	0.80	0.82	0.75	0.75	0.82	0.90	0.90	0.85	0.90	0.70
	Shoot	1.00	1.10	0.90	0.65	0.92	0.88	0.85	0.91	1.10	0.95	1.10	1.20	0.85

1P, 2P and 1/2P are different doses of phosphorus (SSP)

Table 5.5 : Phosphorus contents (ppm) in the root and shoot tissues of pine seedlings at different humidity levels

RH (%)		*L. laccata*			*C. radicata*			*R. luteolus*			*P. tinctorius*			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
High	Root	5.6	3.3	8.5	5.2	3.5	6.6	5.4	4.1	8.8	7.8	3.8	11.6	3.1
	Shoot	5.8	3.6	9.2	4.9	3.7	6.5	5.3	4.5	9.2	8.5	4.1	12.2	4.2
Low	Root	5.5	3.4	6.4	5.0	2.8	6.4	4.4	3.2	6.7	7.9	3.5	10.1	3.4
	Shoot	4.8	2.8	6.1	4.1	3.1	5.7	3.8	3.6	5.8	6.3	3.0	8.6	3.2

RH = Relative Humidity

1P, 2P and 1/2P are different doses of phosphorus (SSP)

Table 5.6 : Nitrogen contents (%) in the root and shoot tissues of pine seedlings at different humidity levels

RH (%)		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
High	Root	0.80	0.82	0.78	0.82	0.75	0.70	0.65	0.85	0.70	0.80	0.76	0.70	0.45
	Shoot	1.00	0.95	1.10	0.70	0.80	0.85	0.92	0.90	0.95	0.92	0.75	1.00	0.61
Low	Root	0.81	0.75	0.80	0.60	0.70	0.75	0.80	0.68	0.70	0.80	0.70	0.95	0.40
	Shoot	0.65	0.55	0.65	0.71	0.65	0.82	0.92	0.60	0.95	0.85	0.68	0.87	0.65

RH = Relative Humidity

1P, 2P and 1/2P are different doses of phosphorus (SSP)

Table 5.7 : Analysis of variance for phosphorus content in pine roots under different light intensities

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of intensities	35.744	2	27.872	39.422*	4.87
Effect of species	37.490	3	12.497	17.675*	3.86
Effect of P levels	92.707	2	46.354	65.563*	4.87
Effect of intensities x species	25.216	6	4.263	5.944*	2.51
Effect of intensities x P levels	2.701	4	0.675	0.955	2.87
Effect of species x P levels	38.206	6	6.368	9.006*	2.51
Error	24.745	12	0.707	0	-
Total	276.810	35	7.909	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 5.8 : Analysis of variance for phosphorus content in pine shoots under different light intensities

Source of variance	SS	DF	MSS	Calculated value	F value at 5%
Effect of intensities	96.161	2	48.080	07.150*	4.87
Effect of species	3.689	3	1.223	1.708	3.86
Effect of P levels	9.107	2	4.554	6.360*	4.87
Effect of intensities x species	20.299	6	3.383	4.725*	2.51
Effect of intensities x P levels	3.026	4	0.757	1.057	2.87
Effect of species x P levels	5.406	6	0.901	1.258	2.51
Error	25.061	12	0.716	0	-
Total	162.729	35	4.649	0	-

* Significant at P = 0.05

P level = Phosphorus level

Table 5.9 : Acid and alkaline phosphatase activity (μ mol p-nitrophenol/100 mg root tissue h^{-1} after 180 days) of ectomycorrhizal roots of pine at different intensities of light

Intensity		*L. laccata*			*C. radicata*			*R. luteolus*			*P. tinctorius*			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
High	AP	10.2	6.5	6.8	4.5	3.4	3.6	4.6	2.3	3.4	9.6	8.5	12.5	10.1
	Al.P	2.9	1.6	1.1	2.2	1.6	1.6	1.2	0.8	1.4	1.2	3.5	1.1	1.8
Moderate	AP	10.2	2.6	12.1	9.1	8.5	10.9	11.5	8.5	6.5	9.2	3.5	4.6	2.6
	Al.P	1.2	0.5	1.4	3.2	1.4	0.5	1.1	0.9	1.1	1.1	0.2	0.1	0.1
Low	AP	3.8	2.5	4.8	6.7	3.0	4.7	4.4	3.7	4.1	7.8	5.2	5.9	2.8
	Al.P	1.2	0.6	0.4	0.2	0.4	0.5	1.1	0.8	0.7	0.7	0.2	0.2	0.1

1P, 2P and 1/2P are the different doses of phosphorus (SSP);

AP = Acid Phosphatase; Al.P = Alkaline phosphatase

Table 5.10 : Acid and alkaline phosphatase activity (µ mol p-nitrophenol/100 mg root tissue h^{-1} after 90 days) of ectomycorrhizal roots of pine at different temperatures

Temp.(°C)		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
25	AP	1.9	1.9	1.9	1.9	2.0	1.9	1.9	2.0	2.1	1.9	1.8	1.9	1.8
	Al.P	0.04	0.02	0.01	7.2	0.01	0.03	0.05	0.02	0.02	0.03	0.03	0.04	0.04
10	AP	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.2
	Al.P	0.1	0.2	0.1	0.2	0.1	0.8	0.2	0.9	0.2	0.2	0.08	0.1	0.2

1P, 2P and 1/2P are the different doses of phosphorus (SSP);

AP = Acid Phosphatase; Al.P = Alkaline phosphatase

Table 5.11 : Acid and alkaline phosphatase activity (μ mol p-nitrophenol/100 mg root tissue h^{-1} after 90 days) of ectomycorrhizal roots of pine at different levels of humidity

RH (%)		L. laccata			C. radicata			R. luteolus			P. tinctorius			Control
		1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	1P	2P	1/2P	
High	AP	0.9	1.2	1.9	1.8	2.1	1.8	1.0	1.9	2.1	0.2	1.1	2.3	2.0
	Al.P	0.05	0.04	0.04	0.05	0.1	0.03	0.05	0.02	0.03	0.04	0.03	0.05	0.02
Low	AP	1.8	1.9	1.9	2.1	1.3	1.2	1.9	1.8	1.8	1.7	1.7	0.9	1.9
	Al.P	0.02	0.03	0.02	0.03	0.03	0.12	0.05	0.04	0.03	0.03	0.12	0.02	0.05

RH = Relative Humidity; 1P, 2P and 1/2P are the different doses of phosphorus (SSP);

AP = Acid Phosphatase; Al.P = Alkaline phosphatase

GENERAL DISCUSSION

GENERAL DISCUSSION

Inverse correlationship between the population of ectomycorrhizal fungi and the altitudes of pine stands is attributed to the high precipitation rate at the higher altitude, which might have leached out the nutrients from the soil and washed out the mycorrhizal propagules resulting in the low population of sporocarpic fungi (Deka et al., 1989).

Soil moisture content was also inversely proportional to the ectomycorrhizal population. Higher moisture content of the soil might have reduced the concentration of available nutrients, hence, reduced the mycorrhizal population.

Soil and atmospheric temperatures were directly proportional to the mycorrhizae and mycorrhizal fungi which was supported by the views of Theodorou and Bowen (1971) that reduction in colonization was due to reduced metabolic activities of fungi.

Positive correlation between ectomycorrhizal fungi and the age of pines has been attributed to the carbon demands of the array of fungi at a time. The statement of Fleming (1983) supported the present findings that young trees may not have excess photosynthate to support the growth of fungi.

Population of mycorrhizae and their sporocarps increased during rainy season. Nitrogen (ammonium) was also higher during this season. Absence of nitrate reductase from the majority of fungi can be attributed to increased uptake of ammonium ions than nitrate ions (Harley, 1969).

Ectomycorrhizal fungi have been reported to be dependent on their host for carbon supply (Snellgrove et al., 1982, 1986) and broad spectrum of fungi utilize soluble and insoluble source of carbon (Palmer and Hacskaylo, 1970). However, the less number of fungi during winter could mainly be assigned to lower temperature inspite of increased amount of organic carbon in the soil.

Less number of fungi at the higher altitude was attributed to the inhibition of phosphate uptake by the fungi due to low temperature (Harley, 1969). Similar correlation was obtained between mycorrhizal fungi and soil potassium.

Among the tested nutrient media (appendix II) MMN's medium was found to be most suitable to promote the growth of fungi at pH 5.5 which was attributed to the easily available carbon source and vitamins in the medium alongwith the acidophilic nature of the ectomycorrhizal fungi (Melin, 1924; Theodorou and Bowen, 1969; Modess, 1941).

Pure mycelial inoculum was produced in large scale for inoculation in experimental practices. Bowen (1965), Mikola (1973), Trappe (1977) and Marx et al. (1980) have proved the pure mycelial inoculum as the most effective method in afforestation programmes.

Several criteria have been used to select ectomycorrhizal fungi for inoculating them in nursery soils (Trappe, 1977). Some of them are temperature, pH, light and relative humidity.

Better growth of most of the fungi at 25°C could be attributed to their improved metabolic activity. The higher temperature may increase the metabolic activity of the fungal mycelia. Better growth of P. tinctorius at 30°C and moderate growth response of C. radicata at 10°C could be attributed to the inter and intra-specific growth variation of mycorrhizal fungi in response to temperature (Hacskaylo et al., 1965).

Improved growth of most of the fungi between 5 to 6 pH range confirmed the opinion of Melin (1924, 1925), Modess (1941), Theodorou and Bowen (1969) and Harley (1969) that most of the fungi grew better on the acidic nature of the liquid medium. Inter-specific growth variation among the test fungi at the different pH levels could be correlated to their differences in selective ion uptake.

In general, the pH of the liquid culture medium was reduced by the mycorrhizal fungi, which suggested that the fungi might either have produced organic acids (Johnston, 1959; Johnston and Miller, 1959; Hung and Trappe, 1983) or differed in selective ion uptake.

Affinity of P. tinctorius for alkalinity confirmed the views of Hung and Trappe (1983) which advocated the acidophilic nature of mycorrhizal fungi with few exceptions.

Comparatively high relative humidity favoured the growth of mycorrhizal fungi which was related to the availability of water for longer period. The study was confirmed by the earlier work of Bowen (1964).

Maximum growth of fungi at 0 lux light intensity contradicted the results of Bjorkman (1942) that the growth of fungi in natural condition was encouraged by the increased light intensity together with the increased quantity of sugar in the root tissue. Such contrasting results could be

explained to the availability of simple carbohydrate *in vitro* conditions compared to symbiotic system, which indicated that the fungi were not directly influenced by the light conditions but were regulated indirectly through the host.

Higher rate of acid phosphatase activity in different mycorrhizal fungi at 30°C could be attributed to their capacity to hydrolyse phosphate ions better and more efficiently at higher temperature (Harley, 1969).

Higher rate of mycorrhizal development at high light intensity than moderate and low light intensity could be attributed to the increased amount of photosynthate available to the mycobiont (Bjorkman, 1942). Regulation of soluble sugars in the root tissue of the host plant might be influenced by the light intensity which promoted better mycorrhizal development under high light intensity than at lower light regimes in nature (Bjorkman, 1942).

Lack of mycorrhizae at low light intensity might be due to unavailability of the fungal auxins to induce the specific physiological and metabolic changes in the roots which are required for the establishment of the symbiotic relationship (Slankis, 1963, 1973). Lack of mycorrhizae at low light intensity can, therefore, be coupled with deficiency of soluble sugars and auxins required to induce the changes in morphology of roots (Slankis, 1951, 1973; Harley and Lewis, 1969; Kozlowski, 1971). Phosphorus levels could not induce the development of mycorrhiza at low irradiance probably due to limited supply of carbon to the mycobiont (Stribley and Snellgrove, 1985; Smith *et al.*, 1986).

Maximum colonization of mycorrhizae by different symbionts was correlated to their adaptability to different temperatures (Hacskaylo *et al.*, 1965; Harley, 1969). The enhanced root metabolism and exudation might have been produced in sufficient quantity, required for the colonization of

mycobiont at 25°C than other temperatures (Bowen, 1970).

Insignificant difference in colonization of pine seedlings at two levels of relative humidity could be attributed to the less difference between the high (70% - 100%) and low (50% - 65%) levels of relative humidity.

Slight increase in seedling growth and mycorrhizal development at 1/2P level under moderate light intensity suggested better carbon supply to the mycobionts at a threshold level of phosphorus.

Enhanced rate of uptake of phosphorus and nitrogen by the mycorrhizal plants than non-mycorrhizal ones under high and moderate light intensity is in conformity with the view of Bjorkman (1942) who advocated that higher light intensity increased the quantity of sugar in the tissue and so encouraged the growth of the fungi that the intensity of infection was also increased. Increased intensity of infection suggested the increased uptake of nitrogen and phosphorus as Hatch (1937) had reported that internal nutrient status was the prime factor in determining the intensity of infection.

Maximum uptake of phosphorus at 2P level under high as well as moderate light intensity contradicted the findings of Bjorkman (1942), who explained that under high light intensity addition of phosphate decreased the mycorrhizal infection, and Son and Smith (1988), who showed positive mycorrhizal response at high irradiance without additional phosphorus. The present findings suggested that 2P level was probably quite low than that of Bjorkman's toxic level.

Uptake of phosphorus and nitrogen was not significantly increased than controls under low light intensity which could be correlated to the Hayman's (1974) view who suggested the reduced phosphorus uptake at low irradiance (Bhat, 1982; Tester et al., 1985; Smith et al., 1986).

Slight increase in phosphorus and nitrogen uptake at 25°C was observed which strengthened the view of Harley (1969) who observed slow uptake of nitrogen and phosphorus at 0°C with subsequent increase upto 15°C to 20°C. Uptake of these elements was not influenced at 10°C by the symbiotic association which again is in agreement with the above view.

Survey of fungal sporocarps revealed the dynamics of the sporocarp occurrence at various altitudes. It is noteworthy that the groups of early and late stage ectomycorrhizal fungi differed at different altitudes. This result suggests that pine forests can be raised more successfully at a particular altitude if those fungi are used as mycorrhizal inoculum which occur dominantly at that particular altitude.

Study on the climatic factors and macro elements suggested that fungal sporocarps can be produced under moderately high temperature, moderate precipitation, moderate humidity and moisture content and low percentage of organic carbon, increased total nitrogen, available phosphorus and potassium.

Study on the climatic factors on the growth behaviour of ectomycorrhizal fungi resulted in many important suggestions.

Optimum temperature for most of the ectomycorrhizal fungi was recorded at 25°C. However, C. radicata and P. tinctorius could grow at 10°C and 30°C temperatures respectively.

High humidity level favoured the growth of L. laccata, C. radicata and R. luteolus while P. tinctorius could grow well at low humidity level.

Dark condition favoured the growth of all the test fungi and increased light intensity reduced the growth.

5-6 pH range was observed optimum for the growth of the ectomycorrhizal fungi, however, R. luteolus and P. tinctorius could grow considerably

at pH-3 and pH-8 respectively.

This study suggested to draw a conclusion that under dark condition and moderately high relative humidity better growth of the ectomycorrhizal fungi could be achieved at 25°C if the pH range of the substrate is 5-6. C. radicata can be exploited in those regions where temperature remains below the optimum range (25°C) and R. luteolus can be used in highly acidic conditions. P. tinctorius can be exploited in diverse edaphic and climatic conditions. Regions having moderately high temperature (around 30°C) and low humidity can be introduced by P. tinctorius as ectomycorrhizal inoculum in afforestation programmes. P. tinctorius can also be used in those areas where soils are highly alkaline.

Study on the isolation, maintenance and mass culture of the ectomycorrhizal fungi showed that isolation of the fungi can be easier from fungal sporocarps than mycorrhizal roots. Better growth of the fungi was obtained on MMN and Hagem's nutrient media suggesting best media for their maintenance and preparation of inoculum.

Study on the effect of light intensity on the development of mycorrhizae concluded that high and moderate light intensities favoured the colonization of mycorrhizae. Colonization of mycorrhiza was observed maximum with P. tinctorius under moderate light intensity whereas under high light intensity mycorrhizal colonization was least by this fungus.

Optimum temperature for mycorrhizal colonization was observed at 25°C, however, low colonization percentage was recorded at 10°C also.

Mycorrhizal colonization has not been found affected by two levels of humidity, however, high percentage of colonization was recorded in seedlings infected by P. tinctorius.

This study suggested that better results in mycorrhizal colonization

can be achieved under high and moderate light intensities at 25°C temperature. P. tinctorius and L. laccata can be exploited for better colonization intensity under optimum range of temperature and light intensity except at high light intensity where infectivity of P. tinctorius is reduced greatly.

Better growth and colonization of pine seedling with C. radicata at 10°C suggests that this fungus can be used in those areas where temperature remains low throughout the year.

Study on the effect of phosphorus fertilization showed that 2P level of SSP can be supplied to those areas where average full day light intensity corresponds to the high light and moderate light intensities, while those areas where average full day light comes in the range of low light intensity, 1/2P level of SSP should be used for the optimum colonization and growth of pine seedlings. Extremely low light intensity can inhibit the colonization of mycorrhizae.

Uptake of phosphorus and nitrogen increased at high and moderate light intensities suggesting that high and moderate light intensities having areas should be chosen for afforestation programmes at an optimum temperature of 25°C and low relative humidity.

SUMMARY

SUMMARY

A study on the seasonal and spatial distribution of ectomycorrhizae and the mycobionts situated at different altitudes of Khasi Hills was done. Fifteen ectomycorrhizal fungi were observed during the whole period of investigation in the vicinity of 2, 7 and 12 years old pine plantations. Maximum number of ectomycorrhizae and their sporocarps were observed at the lower altitude and it decreased with the increase in altitude.

Age of pines influenced the population of sporocarps and ectomycorrhizae which was found to be directly proportional to the age of the stands. Middle rainy season was most conducive for the development of sporocarps. No sporocarp was observed during winter months.

Diversity index of the fungal symbionts was maximum at the lower altitude and minimum at the upper altitude. Boletus sp., Lactarius sp., Russala sp. and Amanita sp. were early successional species at the lower altitude except Lactarius sp. Boletus sp. was found occurring throughout the whole growing season at all the three altitudes.

Positive correlations were obtained between the ectomycorrhizal population and the climatic as well as edpahic conditions in majority of the pine stands at different altitudes. At the higher altitude a negative correlation was obtained between the relative humidity and ectomycorrhizal

population in 12 years old pine stand in the second year of investigation (1988). Similar result was observed between relative humidity and mycorrhizal population in 1988 at the middle altitude in 7 years old stand. Ectomycorrhizae were found negatively correlated with the rainfall in 2 years old stand at the lower altitude in 1988.

Isolation of mycorrhizal fungi, their maintenance, mass inoculum preparation and pure culture synthesis of mycorrhizae with pine (Pinus kesiya Royle ex. Gordon) were done. Pisolithus tinctorius, Scleroderma aurantium, Cenococcum sp., Boletus edulis and Suillus sp. were isolated in pure form and their mycorrhizae were synthesized using these fungi on synthetic media which confirmed their symbiotic relationship with pine roots. Cenococcum sp. was isolated from the mycorrhizal roots while other fungi were isolated from the fungal sporocarps. Digitate and black mycorrhizae were formed by Cenococcum sp. while coralloid type of mycorrhizae were formed by Boletus edulis. Other fungi formed dichotomously branched mycorrhizae.

Pure mycelial inoculum was prepared for their use in experimental purpose.

Effect of climatic conditions i.e., temperature, relative humidity and light intensity and pH were investigated on the growth of Laccaria laccata, Collybia radicata, Rhizopogon luteolus and Pisolithus tinctorius.

Most of the fungi grew well at 25°C but R. luteolus and P. tinctorius were found at 20°C and 30°C respectively.

High humidity level favoured the growth of most of the fungi on solid medium, however, the growth of P. tinctorius was favoured by the low level of humidity.

Dark condition favoured the growth of all the ectomycorrhizal fungi and it decreased with the increase in intensity of light. L. laccata attained maximum diameter followed by C. radicata and R. luteolus while P. tinctorius produced minimum colony spread at lower light intensity.

Most of the fungi grew well between 5 to 6 pH range. R. luteolus and P. tinctorius showed little affinity towards acidic and alkaline conditions respectively.

Acid phosphatase activity of ectomycorrhizal fungi was maximum at 30°C, low humidity, light condition and 3- pH.

Acid phosphatase activity was always more than alkaline phosphatase activity.

Effect of temperature, light and humidity on the colonization of mycorrhizae and uptake of phosphorus and nitrogen by pine seedlings were also studied. High and moderate light intensities favoured the colonization of ectomycorrhizae and efficiency of uptake of nitrogen and phosphorus. However, at 10°C temperature uptake of nitrogen and phosphorus and colonization potential of mycorrhizal fungi were lower.

Relative humidity did not show significant variation in colonization of mycorrhizae as well as uptake of nitrogen and phosphorus. Pine seedlings infected with P. tinctorius attained maximum growth compared to others.

Survival of the pine seedlings was higher under moderate light intensity than high light intensity. Lowest survival of seedlings was observed under low light intensity. P. tinctorius enhanced the survivorship of seedlings more efficiently than other fungi. Uninoculated seedlings showed minimum survival percentage at all the light regimes.

Percentage survival of the seedlings was lowest at 30°C and highest

at 25°C.

Low level of humidity decreased the survivorship of the seedlings compared to high level and it was noticed miminum with L. laccata and maximum with P. tinctorius at low level of humidity.

Different doses of phosphorus affected the growth and mycorrhizal colonization of the pine seedlings. Double dose of the phosphorus (2P) favoured the growth of the seedlings in general at high and moderate light intensities while there was no such effect of 2P level of phosphorus under low light intensity, however, the growth of seedlings infected with R. luteolus was more with 1P level than other levels under high light intensity.

1/2P level of phosphorus was found favourable for mycorrhizal colonization and the growth of pine seedlings at 25°C and 10°C. Insignificant difference in colonization of mycorrhiza and seedling growth was observed between 1P and 2P levels of soil phosphorus. 1/2P level of phosphorus also favoured the intensity of mycorrhizal colonization and growth of pine seedlings at both the himidity levels (low and high).

Survival of the seedlings was also affected by the different levels of phosphorus. Surival of seedlings was reported highest at 1/2P level of soil phosphorus compared to 2P and 1P levels at 25°C and 10°C temperatures. Similar result was observed at low and high levels of relative humidity.

Concentrations of phosphorus and nitrogen were found different in the shoots and roots of pine. Root phosphorus concentration was noticed quite high in 2P supplied set at high light intensity. Seedlings with 2P level were found more efficient in phosphorus absorption under moderate light intensity compared to high light intensity. Uptake of nitrogen was also

more at high and moderate light intensities. No significant difference in the concentrations of phosphorus and nitrogen were observed between the infected and uninfected seedlings at different levels of temperature and humidity.

Alkaline phosphatase activity was found reduced in all the cases than acid phosphatase activity. High and moderate light intensities increased the root phosphatase activity. Similarly enhanced activity of root surface phosphatase was found at 25°C. Insignificant variation in the surface phosphatase activity of the mycorrhizal roots was observed at different levels of humidity.

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* Original not seen.

APPENDIX

Appendix-1 a.

Distribution of pine forests with the year of raising in East Khasi Hills, Meghalaya

Year of raising	RKF	SRF	NB	LK	US	Umkhuti	Shywat	Raitong	Sumer
1954-55	+	-	-	-	-	-	-	-	-
1955-56	+	-	-	-	-	-	-	-	-
1956-57	+	+	-	-	-	-	-	-	-
1957-58	+	-	-	-	-	-	-	-	-
1958-59	-	+	-	-	-	-	-	-	+
1959-60	-	+	-	-	-	-	-	-	-
1960-61	+	-	-	-	-	-	-	-	-
1961-62	+	+	-	-	-	-	-	-	-
1962-63	+	+	-	-	-	-	-	-	-
1963-64	+	-	-	-	-	-	-	-	-
1964-65	+	-	-	-	-	-	-	-	-
1965-66	+	-	-	-	-	-	-	-	-
1966-67	+	-	-	-	-	+	-	-	+
1968-69	+	-	-	-	-	-	-	-	-
1969-70	+	-	-	-	-	-	-	-	-
1970-71	+	-	-	-	-	-	-	-	-
1971-72	+	+	-	-	-	-	-	+	-
1972-73	+	-	-	+	-	-	-	-	-
1973-74	+	-	-	+	-	-	-	-	-
1974-75	+*	-	+*	+*	-	-	-	-	-
1975-76	+	-	+	-	-	-	-	-	-
1976-77	+	-	+	-	-	-	-	-	-
1977-78	+	-	+	+	-	-	-	-	-
1978-79	-	+	+	-	-	-	+	-	-
1979-80	+*	-	+*	+*	-	+	+	-	-
1980-81	+	-	+	-	-	+	-	-	-
1981-82	+	-	+	-	+	+	-	-	-
1982-83	-	-	+	-	+	-	-	-	-
1983-84	-	-	+	-	-	+	-	-	+
1984-85	+*	-	+*	+*	-	-	-	-	+
1985-86	+	-	+	-	+	-	-	-	-

RKF = Riat Khwan Forest; SRF = Short Round Forest; NB = Naya Bunglow forest; LK = Laitkor forest; US = Upper Shillong forest;

+ = Presence; - = Absence; +* = Selected sites for the present study.

Appendix 1b

List of ectomycorrhizal fungi observed during the course of investigation period

Sl. No.	Investigation period	
	1987	1988
1.	Amanita sp.	Amanita sp.
2.	Boletus sp.	Boletus sp.
3.	Collybia sp.	Clitocybe sp.
4.	Elaphomyces sp.	Cortinarius sp.
5.	Gomphidium sp.	Elaphomyces sp.
6.	Hygrophorus sp.	Gomphidium sp.
7.	Lactarius sp.	Hygrophorus sp.
8	Pisolithus sp.	Lactarius sp.
9.	Scleroderma sp.	Pisolithus sp.
10.	Suillus sp.	Russala sp.
11.	Tricholoma sp.	Scleroderm sp.
12.	-	Suillus sp.
13.	-	Terferia sp.
14.	-	Tricholoma sp.

Appendix-Ic

Different ectomycorrhizal fungi collected during the whole period of investigation

G_1 Amanita sp.

G_2 Boletus sp.

G_3 Clitocybe sp.

G_4 Cortinarius sp.

G_5 Elaphomyces sp.

G_6 Gomphidium sp.

G_7 Hygrophorus sp.

G_8 Lactarius sp.

G_9 Pisolithus sp.

G_{10} Russula sp.

G_{11} Scleroderma sp.

G_{12} Suillus sp.

G_{13} Terfezia sp.

G_{14} Tricholoma sp.

G_{15} Collybia sp.

LIST OF CULTURE MEDIA USED DURING THE COURSE OF INVESTIGATION

Peptone Dextrose Rose Bengal Agar (Martin, 1950)

Agar, 20 g; KH_2PO_4, 1 g; Mg SO_4 $7H_20$, 0.5 g; Peptone, 5 g; Dextroxe, 10 g; Rose Bengal (1%), 3.3 ml, distilled water, 1000 ml; Streptomycin, 30 mg.

Nutrient Agar (Difco Manual, 1953)

Agar, 15; Beef extract, 5 g; Peptone, 5 g; NaCl, 8 g; distilled water, 1000 ml. After autoclaving pH adjusted to 7.3.

Starch Casein Agar (Kuster and Williams, 1964)

Agar, 18 g; Starch, 10g; Casein (Vitamin free), 0.3g; KNO_3, 2 g; NaCl 2 g; K_2HPO_4, 2g; Mg $SO_4.7H_2O$, 0.5g; $CaCO_3$, 0.02g; $FeSO_4$ $7H_20$, 0.01g; distilled water, 1000ml, Nystatin and actidione, 50 ug/ml of each. After autoclaving pH was adjusted to 7-7.2.

Hagem Agar (Modified by Modess, 1941)

Agar, 20 g; Glucose, 5 g; Malt extract, 5 g; KH_2PO_4, 0.5 g; Mg SO_4 $7H_2O$, 0.5 g; NH_4Cl, 0.5 g; $FeCl_3$, 0.1 ml (1%); distilled water 1000 ml. pH adjusted to 5.5-6 after autoclaving.

Melin-Norkran's Nutrient Medium (Modified, Marx, 1969a)

Agar, 15 g; $CaCl_2$, 0.05g; NaCl, 0.02g; KH_2PO_4, 0.5g; $(NH_4)_2$ HPO_4, 0.25 g; Mg SO_4 $7H_20$, 0.15 g; $FeCl_3$ (1%) 1.2 ml; Thiamine HCl, 100 ug; Malt extract (paste) 3g; distilled water, 1000 ml. After autoclaving pH was adjusted to 5.5-5.7.

Malt Extract Agar Medium (Raper and Thom, 1949)

Malt extract, 20 g; Dextrose, 20 g; Peptone, 1 g; Agar 20 g; distilled water, 1000 ml. Medium autoclaved at 15 lb/inch for 15 minutes.

Czapec's Dox Agar Medium (Raper and Thom, 1949)

Agar, 15 g; $NaNO_3$, 3 g; K_2HPO_4, 1 g; Mg $SO_4 7H_2 0$, 0.5g; KCl, 0.5 g; $FeSO_4$, 0.01 g; Sucrose, 30 g; Yeast powder, 1 g; distilled water, 1000 ml. After sterilization pH was adjusted to 7.0.

AT Agar Medium (Pachlawska and Pachlewski, 1974)

Agar, 15 g; Thiamine, 100 ug; distilled water, 1000 ml. Following to autoclave the pH of medium amounted to 5.5-6.0.