Chapter - 2

Review of Literature
Review of Literature.

The root system:

Trees have usually perennial root system consisting of a mixture of roots of different types and ages (Atkinson, 1974). The individual tree species posses distinctive root system which is characterised by a definite root habit i.e. form, direction and distribution of small roots (Bilan, 1971). He pointed out that the overall growth and development of root system is under the control of both heredity and environment, the former determining the initial root growth and the latter affecting the subsequent development of the root system.

Atkinson (1980) reviewed the growth and development of roots in fruit trees. According to Rogers (1939a) "The root system" is generally more extensive than the branch system and in trees it may occupy soil layers extending to many feet below the surface. The root will in fact, grow in any soil area which supplies what it requires, subject to limiting factors. The mature fruit tree root system is reported to include roots that differ in age, diameter and degree of suberization. The young root is initially white and bears short root hairs.

Rogers and Head (1969) classified apple roots into extension and lateral roots. The thick extension roots survive while the thinner lateral roots tend to be short lived, although this class of root can be infected by mycorrhizal fungi (Mosse, 1957) Two types of root system viz. scaffold roots, which are long and thick; and
fibrous roots which are short, 0.3mm thick, have been described by Kanwar (1987). These roots can also be named as horizontal roots, which are more or less parallel to the surface, and vertical roots which grow straight downward, going as deep as 6 to 12m. The scaffold roots, which may be vertical or horizontal produce the fibrous roots of the plants.

The vertical roots are the anchorage roots which hold the tree in its place. The main vertical root which penetrates deep into the ground and has very few laterals is called the tap root. Horizontal roots spread out over a large area particularly where microorganisms are most active. They spread farther than the branches by one and a half or two times the length of the branches. This ratio is established during the second year of the plants life and remains practically constant thereafter. This is true not only for seedling and young tree but for all trees, irrespective of variety, root stock age and local conditions. The fibrous roots may be grouped as (i) growing; (ii) associating or active; (iii) intermediate, and (iv) conducting.

Head (1970) had shown that under some circumstances roots may become highly branched and remain brown for several years without any further development. The young root is initially white and succulent with short root hairs. After one week to four weeks of development it begins to turn brown and the root hairs shrivel (Rogers, 1939b). Browning takes an average of 2 to 3 weeks during winter (Head, 1966). The browning spreads as a wave from older
regions towards the tip. It is assumed that the absorption of water and minerals occur exclusively through younger parts of the root system i.e. root tips and root hairs (Kolesnikov, 1971). But in case of tree crops, all roots rather than those newly produced are apparently effective to small extent.

The Mycorrhiza:-

More than a century of observation and experiments with root system of the plants from natural habitat have shown that a symbiotic relationship between tree roots and fungi form definite composite organ called mycorrhiza which vary in structure and composition according to species of host and fungus and to the condition of the habitat. This intimate association between the fungus and tree roots is no longer regarded as uncommon in nature but rather as the general rule; the plants, woody, herbaceous including many crop plants, both monocotyledons and dicotyledons are now known to regularly possess this symbiotic association. It has been estimated that about 95 percent species of vascular plants in the world are mycorrhizal (Trappe, 1977).

Frank (1885) introduced the term mycorrhiza (literally meaning the fungal roots) to describe a structure that results from a mutually beneficial association between the fine feeder roots of the plant and species of highly specialized root inhabiting fungi. According to Wilhelm (1966) under field conditions the plants do not strictly speaking have roots, they have mycorrhizae. With the exceptions of aquatic plants, some halophytes and
few other plants in the world, all form mycorrhizae in natural roots to varying degrees (Gerdemann, 1968; Malloch et al; 1980). Therefore it is easier to name the plant groups in which these associations do not occur or have yet to be reported viz. the order Centrospermae and the family Cruciferae, Cyperaceae, Fumariaceae, Commelinaceae, Urticaceae, Polygonaceae (Gerdemann, 1968). However Gerdemann (1975) has cited exceptions where endomycorrhizae were found for several members of the order Centrospermae, family Chenopodiaceae (Ross and Harper, 1970); Kruckleman, 1973, 1975). Most form of the mycorrhizae are immensely beneficial to plants especially in case of Pinus, it is indispensable to its growth.

Most research of mycorrhiza has been aimed at demonstrating that the host plant is benefitted by improved mineral nutrition enhanced growth and protection against diseases (Wilson, 1977). The fungal symbionts receive in turn a supply of essential carbohydrates and other organic metabolites from the host plant (Gerdemann, 1968, Harley,1969). Fungal species vary widely in their number of potential hosts and hosts vary from broadly acceptive to highly restrictive in their possible fungal associates (Molina and Trappe, 1982a; Molina and Trappe, 1982b).Studies of Robertson (1954) on Pinus sylvestris. L. revealed that mycorrhizal fungi may also colonize long roots. Similarly out of intensive and extensive root systems recognised by Busgen (1901), the former is known to possess both ecto and endomycorrhizae where as the latter only endomycorrhizae.
Types of mycorrhizae:

Plenchette (1982) reported seven kinds of mycorrhizae namely ectomycorrhiza, endomycorrhiza, ectendomycorrhiza, orchidaceous, ericaceous, arbutoid and monotropoid mycorrhiza, which were later confirmed by Harley and Smith (1983). On morphoanatomical basis mycorrhizae fall into three different groups viz. Ectomycorrhizae, Endomycorrhizae, and Ectendomycorrhizae.

Ectomycorrhizae:

This kind of mycorrhiza with its conspicuous external sheath of fungal tissue is called ectotropic mycorrhiza. The hyphae which penetrate the cortex form a network known as ‘Hartig net’ in the middle lamella of the cell wall, and the intercellular spaces of the external cortex. Hyphae pass outward into the soil and ramify among the soil particles. These may be scarce or abundant in different kinds of mycorrhizae. Roots are often surrounded externally by a fungal sheath, the fungal mantle. Ectomycorrhizal fungi outnumber their hosts. An estimated, 5000 fungi can establish ectomycorrhizae with about 2000 woody hosts. In north America alone about 2100 species of fungi are estimated to form ectomycorrhizae with forest trees. Trappe (1962) comprehensively listed fungi along with host plants. Among the basidiomycetous fungi, the majority are members of hymenomycetes, particularly in the families, Boletaceae, Gomphidiaceae, Russulaceae, Strobilomyctaceae, Cantharellaceae and genera Amanita, Armillaria,
Astreasus, Suillus, Tricholoma, Laccaria and Lactarius and in Gastromycetes: Rhizopogon, Scleroderma and Pisolithus.

Species of plants known to form ectomycorrhiza belong mainly to the families Pinaceae, Betulacea, Fagaceae, Myrtaceae, subfamily Ceasalpinoideae and Diptercarpaceae (Harley, 1969; Malloch et al., 1980) as well as many members of the Rosaceae, Leguminosae and Ericaceae (Meyer, 1973). Ectomycorrhiza is a fairly balanced Symbiosis which proves advantageous to both partners. Ectomycorrhizae actually represent absorptive organs of tree and that the hyphae spreading from the fungal sheath into the soil act in an analogous manner to root hairs in salt absorption (Meyer, 1974). Ectomycorrhizal association has higher phosphotase production potential than other mycorrhizae (Dighton and Coleman., 1992).

Morpho-anatomical features of ectomycorrhizae:

Ectomycorrhizae are morphologically distinct and can be recognised due to heterorrhizic root system comprising of two kinds of roots; long roots of potentially unlimited extension and short roots with restricted growth and life span. Infected short roots become swollen and variously coloured due to the colour of fungal symbionts. The uninfected root apices either maintain very active growth or abort. Mycorrhiza develops as lateral outgrowth from the mother root. As per the branching, the ectomycorrhizal roots may be simple or monopodial as in spruce and fir (Bakshi, 1957) coralloid as in deodar (Bakshi, 1957) repeatedly dichoto-
mous; only branched as in pines (Bakshi and Thapar, 1960, 1966, Bakshi et al., 1968; Mukherjee and Rehill, 1962). Each short root regardless of branching pattern is an ectomycorrhiza. Functionally when ectomycorrhizae are formed the absorbing area of root surface is increased (Harley, 1959); Root hairs are absent and these roots are completely enveloped by a fungal sheath.

Mycorrhizal structures are not static organs but change ontogenetically from a juvenile to a mature state and finally undergo senescence (Atkinson, 1975; Harley and Smith 1983; Harley, 1968). Mycorrhizae may become inactive and may be lost by attrition alternatively, they may undergo cycles of dormancy and active growth which is a common feature of perennial root system of trees (Wilcox, 1968a,b Kottke et al. 1986).

**Mantle:**

A mat of fungal tissue which develops on the surface of mycorrhizal roots encloses completely the host root having variable thickness, texture and colour is called "Mantle." Thickness of fungal mantle varies from 1-2 hyphal diameter to as many as 30-40, depending on the fungal associate, host and environmental conditions (Marx, 1972). Mantle surface can range from thin to profuse and texture can vary from smooth, velvety, warty to granular (Zak, 1973). The hyphae radiating from the mantle surface may be simple or branched, bearing simple or clamped septae. Colour of these hyphae may be hyaline, black or in various shades as orange, yellow or brown. The colour of mantle is mainly due to
the colour of radiating hyphae. Mantle may be smooth or with radiating hyphae which enter into soil to increase the absorbing surface of root.

Foster and Marks (1967) showed that the cells of the mantle hyphae were covered with amorphous layer which on mild maceration revealed few layers of microfibrils. The inner being more organised than the outer as in fungi of many groups (Arson and Preston, 1960; Hawker, 1965). The inner layer of mantle was characterised by more closely interwoven hyphae (Foster and Marks, 1966) and an increase in the number of cytoplasmic organelles (Scannerini, 1968). Chilvers (1968b), Dominik (1959) and Zak (1973) have shown that it is possible to recognise and identify mycorrhizae by structural peculiarities of hyphal mantle. When the hyphae comprising the mantle can no longer be recognised, it is then called a pseudoparenchyma; or synechyma (Dominik, 1959; Chilvers, 1968b; Godbout and Fortin, 1985). The synechymatous mantle structures may have approximately isodiametric cells with fairly straight walls or puzzal like cells with many walls. The inter hyphal spaces may be larger or cemented with a matrix from the slimed hyphal walls. But when hyphae maintain their identity and can still be recognised, the structure is called plectenchyma or prosenchyma (Dominik, 1959; Godbout and Fortin, 1985). The characteristics of hyphal mantle can best be recognised in tangential sections (Haug et al., 1986).
Hartig Net:-

The "Hartig Net" has been considered the hallmark of the ectomycorrhizal association (Mikola, 1965) and much attention has been devoted to its development (Clowes, 1951; Wilcox, 1968a; Marks and Kozlowski, 1973; Nylund and Unestami, 1982). When the Hartig net development is initiated the cortical or epidermal cells have obviously already acquired their final dimensions (Massicotte et al., 1986; Warren et al., 1983).

During the mature stage of mycorrhizal structure cortical cells become enveloped more or less completely by a living hyphal system. The dormant stage shows the root cap cells and cells lying in between the endodermis and root cap becomes suberized and accumulate polyphenols (Wilcox, 1954; Kottke et al., 1986). Thus the apical meristem is enriched by a protective layer and mycorrhizal fungi are barred from the meristem during dormancy (Kottke and Oberwinkler, 1986a). The dormant mycelium may regrow slowly or rapidly after dormancy. In the former the root tip does not break through the sheath, but both host and fungal tissues may grow slowly in unison (Harley and Smith, 1983), in the latter the root apex breaks through the hyphal sheath (Wilcox, 1968b). The process of Hartig net development was reviewed by Kottke and Oberwinkler, (1986b).
Endomycorrhiza (Vesicular-Arbuscular mycorrhiza):-
No coherent sheath is usually formed by the fungus outside the roots, although there may be a loose weft of hyphae in the soil around the root. Hyphae penetrates cortical cells of the feeder roots (Carling and Brown, 1982). The VAM fungi form large vesi-
cules in the cortical tissues (Mosse, 1973). Vesicular and Arbuscular mycorrhizal fungi form resting spores, which are born either singly in soil or in hypogeous (or rarely epigeous) sporo-
carps. The spores range from less than 15 um in diameter (Glomus tenuis) to over 500 um in diameter (Gigaspora gigantea). Under favourable conditions spore germination takes place. Germ tubes grow through soil and may eventually come in contact with roots of suitable host. Appresoria are formed and hyphal penetration into or between epidermal cells takes place. Hyphae grow through the epidermis and into the cortex, where ramification of hyphae is both inter and intracellular. Tissues medial to the cortex are not invaded nor are epidermal cells after initial penetration. Longitudinal spread of infection appears to be limited to approx­imately 5mm around a single penetration point (Cox and Sanders, 1974).

Ectendomycorrhizae:-
Ectendomycorrhizae resemble ectomycorrhizae in forming Hartig net and a fungal mantle but also resemble endomycorrhiza in intracel-
lar penetration of cortical cells (Wilcox, 1971). The term "ectendomycorrhizae" was orginally used by Melin (1923) for roots with a combined intra and intercellular infection. He was of the
opinion that the association was mutualistic. However, many subsequent investigators interpreted these associations as being parasitic and others termed it pseudomycorrhizal. Mikola (1965) resolved the confusion between pseudomycorrhizae and ectendomycorrhizae and clarified the nature and occurrence of the latter. He reported the prevalence of ectendomycorrhiza in coniferous nurseries infecting *Pinus* and *Larix* characterised by little or no fungal mantle and large diameter inter cellular hyphae, heavily ramified throughout the cortex of both long and short roots. In ectendomycorrhizae mantle sheath is much reduced or even absent, and the ‘Hartig Net’ is usually well developed but the hyphae also penetrate into the cells of the host. Pure culture synthesis of ectendomycorrhiza in aseptic conditions has been successful (Wilcox and Ganmore, 1974).

**Mycorrhizal functions** :-
It is now well established that free seedlings without mycorrhizae fail to grow in soils low in nutrients but establish successfully if inoculated with suitable mycorrhizal fungi (Harley 1959). A number of functions have been assigned to mycorrhizae that afford benefit to trees. Most of the benefits accrued are known to be accomplished through physiological processes. Even of greater importance than this is the fact that the ectotrophic mycorrhizal roots are less susceptible than non-mycorrhizal roots to infection by root pathogens. Zak (1964) postulated that mycorrhizal fungi may protect absorbing roots of
trees by; (i) utilizing root carbohydrates and other chemicals thereby reducing the attractiveness of the root to pathogens. (ii) providing a mechanical barrier to the pathogen in the form of the fungal mantle (iii) secreting antibiotics which may inhibit or kill potential pathogens; and (iv) attracting while in mycorrhizal association with the host root, a protective rhizosphere population of other microorganisms.

Plants are being helped by mycorrhiza in absorbing many minerals from the soils more efficiently than non-mycorrhizal plants, e.g., Calcium, Potassium, Copper, molybednum, magnesium and zinc (Hatch, 1937; Mejestrik, 1970; Bowen et al., 1974). The fungal sheath can store inorganic nutrients e.g. chloride (Smith, 1972), ammonium (Carrodus, 1967) and especially phosphate (Harley and McCready, 1952 a,b) and release them to the plants during periods of deficiencies/or active growth. Among the different major and minor elements, the uptake of phosphorus has been well established (Hatch, 1937; Melin, 1953; Harley, 1959). In containerized plants, Rhodes and Gerdemann (1975) demonstrated that ectomycorrhizal symbionts increased the phosphate depletion zone to about 7mm whereas normally the phosphate depletion zone is 1mm to 2mm wide. Accumulation of phosphate in mycorrhizal roots has been demonstrated in pine by studying absorption of $^{32}$P labelled phosphate (Kramer and Wilbur, 1949; Melin and Nilsson, 1950). Morrison (1957, 1962) observed a steady movement of phosphate ions in the shoots of mycorrhizal plants of P. radiata whereas there was little translocation in non-mycorrhizal seedlings. Mejestrik
and Krause (1973) and Herrera et al. (1978) provided direct evidence for utilization of organic phosphorus sources by mycorrhizal by using radiotracer technique. Although the work of Herrera's group was non-quantitative, they demonstrated by autoradiography uptake of 32p-labelled phosphate into fungal and root material from labelled leaves. Mejestrik and Krause (1973) used labelled humic organic phosphate (32p immobilized in fungi and bacteria) and showed that *Cenococcum geophilum* and *Suillus luteus* mycorrhizal roots took up twice and four times as much label respectively as non-mycorrhizal roots. Thomas et al. (1982) using radiotracer could not detect decomposition of complex on organic phosphate formed by *T. terrestris* associated with Sitka spruce. Various workers (Crosset and Loughmann, 1966; Gianinazzi and Gianinazzi, 1986; Harley and Brierlay, 1955; Cox et al., 1975; Callow et al. 1978 and Ling-lee et al., 1975) confirms that phosphorus accumulates as polyphosphates metachromatic lead staining granules, (Strullu, 1982) which get remobilized when required (Harley et al., 1953; Martin et al., 1986). It is not very clear whether the mycorrhizae are able to exploit phosphate in the soil not available to non-mycorrhizal roots or whether mycorrhizal roots are just more efficient in capturing the available soluble phosphates (Kormanik et al., 1977). Nevertheless, some studies have indicated the ability to absorb normally insoluble phosphate sources through presence of phosphate enzymes on mycorrhizal surface (Maronek et al., 1981). The evidence for the induction of phosphotase activity in response to the lack of inorganic phos-
Phosphate by ectomycorrhizal fungi have been given by various workers (Alexander and Hardy, 1981; Bousquet et al., 1986; Calleja et al., 1980. Coupe et al., 1982; Kroehler et al., 1988; Mousin et al., 1988; Mousin and Salsac, 1982.)

Dighton (1983) concluded that more inorganic phosphate was produced than required for fungal growth indicating a source of phosphorus for the host plant. Phosphatase production by ectomycorrhizal fungi in pure culture has been demonstrated by a number of authors (Antinus et al., 1986; Bousquet et al., 1986; Calleja and Auzac, 1982; Calleja et al., 1980; Coupe et al., 1982; Dighton, 1982 Healy and Dighton, 1986; Ho. I and Zak, 1979, Kroehler et al., 1988; Mousin and Salsac, 1982; 1971). Despite the overwhelming evidence for phosphatase producing potential by ectomycorrhizal fungi in culture, there is a need to explore the activity of these enzymes in intact mycorrhizal and to look for their production not at the root surface but in the distal hyphae at the site of resource capture (Dighton, 1991).

There has been little study on the absorption of substances other than nitrogen and phosphorus by ectomycorrhizal either from the soil or from solution. Literature on nitrogen nutrition of mycorrhizal plants have been reviewed by Bowen and Smith (1981), Alexander (1983), Reven et al., (1978). Ectomycorrhizal fungi such as Suillus bovinus, Amanita muscaria, Paxillus involutus and Rhizopogon reseolus have been shown to use peptides and proteins as nitrogen sources in both pure culture of the fungus and in
association with *Pinus contorta* (Abuzinadah et al., 1986 a.b). Harley and Smith (1983) reported that potassium is readily lost from mycorrhizal roots. It is present in the tissues at about 70 mole per 100 gm dry weight or if assumed to be distributed in the total tissues water at about 0.1M. Losses occur when the tissue is kept at low O2 concentration or when kept about 20°C in normal O2 supply. Edmonds et al., (1976) observed that addition of Glucose to beech mycorrhiza leaking potassium (K) at 20°C caused a rapid cessation of the leak. The differential uptake of these elements however is not normally reflected in plants growth (Gray and Gerdemann, 1973).

Meyer (1974) suggested some reciprocal relationship between the fungus and the plant host in which it was difficult to interpret the exact contribution of either organism. The mycorrhizal development increases the surface area of roots and thereby the nutrient and water uptake. The ectomycorrhizae are reported to absorb and accumulate N, P, K and Ca in fungal mantles more rapidly and for longer periods of time than non-mycorrhizal feeder roots. They are also known to break down certain complex mineral and organic substances in the soil and transmit nutrients from these material to the tree. The mycorrhizae also appear to deter infection of feeder roots by root pathogens and increase the tolerance of trees to drought, high soil temperatures, soil toxins and extremes of soil pH caused by high levels of S and Al (Marx, 1977). Maronek et al., (1981) summarised the interactions
between the host and fungus during mycorrhizal development. They
have mentioned these interactions very complex, which "appear to
be influenced by a myriad of interrelated biochemical, physio-
logical and environmental processes. Mycorrhizae are known to
reclaim and increase fertility of soils, which are poorly man-
aged, continuously disturbed and regularly mined. Among the
various major and minor elements the uptake of P has been well
documented (Hatch, 1937; Melin, 1953; Harley, 1959). The mycorr-
hizal development in relation to fertility levels is influenced
by a number of factors such as host specificity, ecotypes, soil
characteristics, initial inoculum density, pH, seasonal changes
and moisture levels (Maronek et al., 1981).

Mycorrhizal infection decreases with increasing levels of P in
the soil or growth medium (Daft and Nicolson, 1969, 1972; Mosse
and Philips, 1971; Mosse, 1973b; Hall, 1975). Consequently, at
very high soil P levels, only a small fraction of the root
system may be infected. It is the concentration of the P in the
plant tissues, rather than P in soil, that regulates the degree
of mycorrhizal infection. Sanders (1975) found that foliar appli-
cation of P to onion plants resulted in decreased mycorrhizal
infection. Similar result was obtained by Menge et al., (1978)
who used a split root system technique. They found that when one
half of a sudangrass (Sorghum sudanense) root system grew in a
high P soil, mycorrhizal infection was reduced in the other half
of the root system even though it grew in a separate container of
relatively low P soil.
There is increasing evidence that P is translocated in the form of Polyphosphate. Bodies were found in the hyphae of VA fungi which gave metachromatic reaction with toluidine blue stain, a characteristic of polyphosphate (Cox et al., 1975) and (Ling et al., 1975). On the basis of this evidence and other histochemical test, they suggested that these bodies were granules of polyphosphate. The faint staining of vacuolar contents indicated the presence of polyphosphate granule in each fungal vacuole (Cox et al., 1975). Vacuoles could be seen to move rapidly with the cytoplasmic flow in the fungal lumen. Consequently, it was suggested that polyphosphate could serve as an efficient means of P translocation by external hyphae (Cox et al., 1975; Tinker, 1975). Strullu (1982) confirmed that phosphorus accumulates as polyphosphates, metachromatic lead staining granules which get remobilised when required (Harley, et al., 1953; Martin et al., 1986).

In various horticultural crops the increase in P concentration following endomycorrhizal inoculations has been reported (Hughes et al., 1979; Kleinschmidt and Gerdmann, 1972). Marx (1971) observed that the results from studies on increased uptake of the other major and minor elements by mycorrhizal plants have been even more variable than those concerning phosphate. Sometimes the elements N, K, Ca, Mg, Fe, Cu, Mn, Na, Si, Zn, Al and B are present in greater concentrations in mycorrhizal plants than in non-mycorrhizal plants. In other cases the concentration of these
elements is higher in non-mycorrhizal plants and sometimes no significant difference in concentration is observed. Harley and Wilson (1959) observed that K is observed readily and accumulated in the sheath. Whereas Ca was found in polyphosphate granule (Strullu, 1982 and Strullu et al., 1983). Clement et al. (1977) have shown that on calcareous soils the excessive uptake of K and Ca is reduced by mycorrhization. Investigations of Rygiewicz et al. (1984) and Bledsoe and Rygiewicz (1986) pointed out that the high rates of ammonium uptake decreases uptake of K, Ca and Mg in several mycorrhizal mycelia. It has often been reported that certain micronutrient deficiencies, particularly of Zn and copper (Cu), result from high P fertilization (Olsen, 1972).

The carbohydrate transfer from the host to the fungus is presumed to be reciprocated by the transport of N, P, K and Ca by the fungus to the host (Melin and Nilsson, 1955; Dexheimer et al., 1982; Harley and Smith, 1983; Dexheimer et al., 1986). The form in which C is transferred from host to endophyte is not known. In ectomycorrhizae, sucrose appears to be the principal carbohydrate transferred from host to fungus. Sucrose is rapidly converted to trehalose and mannitol, and eventually to glycogen, which is stored by the fungus (Lewis and Harley, 1965; Bevege et al.; 1975). Neither trehalose nor mannitol has been detected in VA mycorrhizae (Hayman, 1974; Bevege et al., 1975). Although glycogen in hyphae of a mycorrhizal fungus contained approximately 10% of the total C label extracted from fungal material after exposure of tops to C, a larger proportion (56.2%) was found in metal
precipitated fraction, suggesting storage as lipids or lipoproteins (Bevege et al., 1975). This result agrees with histochemical results (Cox et al., 1975; Cooper and Losel, 1978) that show a large proportion of the fungal volume occupied by lipid. Cooper and Losel (1978) found G. Mosseae mycelium to contain 43.8% lipid. Triglyceride, diglyceride, and free fatty acids predominated the neutral lipid fraction and phosphatidyl ethanolamine was the principal phospholipid present. The mycorrhizal fungi assimilate soluble carbohydrates and hence the degree of mycorrhizal development depends upon the quality of soluble sugar in the roots (Bjorkman, 1970; Hacskaylo, 1971).

Maronek et al. (1981) pointed out that although the production of auxins, cytokinins, gibberellins vitamins by mycorrhizal fungi in pure culture and their effect on plant growth and development are well documented, none has yet shown that any mycorrhizal fungus produces a growth hormone while in association with the root. Synthetic auxins have been shown to influence the induction of mycorrhizal structures on the roots of Pinus sylvestris L. and P. strobus L. and when auxin treatment was discontinued, the mycorrhizal characteristic of the root were also found to disappear. Activity similar to that induced by auxin has been reported with Colchicine, Kinetin and various vitamins (Slankis, 1975). Marx et al. (1977) substantiated the findings of Bjorkman (1942) that low nitrogen content of the soil induces greater mycorrhizal development in roots. The agricultural plants
have been reported to use nitrates as N source compared to ecto-
mycorrhizal plants which use ammonium (Melin and Nilsson, 1953; Bledsol and Rygiewicz, 1986). The ability of forest trees to take up ammonium is important in soils with low pH and low nitrification rates. The large surface provided by hyphae and hyphal strands enables the mycorrhizae to take up sufficient amounts of rather immobile ammonium (Kottke and Oberwinkler, 1986a).

The mycorrhizal fungi are known to confer some resistance to disease in plants. Mycorrhizal fungi have also been reported to produce certain antibiotics, though their production and identification are still not well documented (Marx, 1972; Marx and Davey, 1969). Mycorrhizal roots are also known to survive better under conditions of stress compared to non-mycorrhizal roots. Experiments conducted on tolerance of stress by seedlings of fir (Abies pindrow Royle), spruce (Picea smithiana Boiss) and chilgoza pine (Pinus gerardiana wall) revealed that mycorrhizal seedlings of these tolerated higher stress levels compared to non-mycorrhizal seedlings (Lakhanpal and Sharma, 1988). Different species of mycorrhizal fungi have been shown to exhibit varying degrees of drought tolerance under conditions of moisture stress (Worley and Hacskaylo, 1959; Trappe, 1962). Many mycorrhizal fungi possess specific individual traits with respect to tolerance to soil temperature extremes, pH, moisture, low fertility, salinity and toxicants etc., which many provide the host plant with an ecological competitive advantage facilitating
increased plant survival, growth nutrition and/or yield under stress conditions (Trappe, 1977). Some *Glomus* species have been reported to be adapted to higher soil temperature than other (Schenck and Schroder, 1974; Schenck et al., 1975). Extra material mycelium of VAM fungi has been shown to be important in the binding of sand grains and thus in the stabilization of dunes and sandy soils (Clough and Sutton, 1976; Nicolson and Johnston, 1979). Development of soil aggregates of about 2mm diameter is also dependent upon binding by mycorrhizae hyphae (Tisdall and Oades, 1979). Mycorrhizae may therefore have an important role in the development of soil structure which is independent of any effect, upon growth of individual plant species.

**Sources of Inoculum:**

 Attempt to use specific fungi to form mycorrhizae on seedling dates back to the 18th century (Trappe, 1977). Sporophores of truffles fungi were added to planting holes of oak seedlings in new plantations in attempts to enhance Truffle production (Malencon, 1938). From time to time many workers Marx and Cordell, 1989; and Marx et al., 1991) have discussed the different inoculation methods and various types of inocula used for nursery inoculations.

Most of the ectomycorrhizal fungi produce numerous spores and can be disseminated by wind, rain, insects, small animals and man to
the surrounding seedling producing nurseries where they rapidly colonise the seedling roots. The most easiest and commonly used source of ectomycorrhizal inoculum is soil humus or duff containing mycorrhizae and associated mycelium. In spite of the drawback that the specific fungi in the mixture can not be controlled, it is the most reliable method of eliminating mycorrhizal deficiencies and is used by nursery men regularly (Mikola, 1970; Molina, 1977). Another method of artificial inoculation is by planting mycorrhizal "nurse" seedlings or excised mycorrhizae for new seedling crops. Through this method also there is possibility of the introduction of unwanted pest and pathogens (Mikola, 1970, 1973). The third method is the use of spores on crushed sporocarps as inoculum (Molina, 1977). However spore inoculum takes several weeks to form mycorrhizae. This infection process is slow in comparison to the mycelium inoculum (Marx et al., 1976; Theodorou and Bowen, 1970).

The use of pure mycelial cultures of ectomycorrhizal fungi has been repeatedly recommended as the most biologically ideal method of inoculation (Bowen, 1965; Marx, 1977; Mikola, 1973; Shemakhanova, 1962; Trappe, 1977). Though it is cumbersome and time consuming for large scale field inoculation. Technique for wide scale application have now been developed (Marx, 1981). Initially the pure culture of ectomycorrhizal fungi is obtained either from the fruit bodies or from the ectomycorrhiza itself. The isolation from sporocarp is however, easiest than ectomy-
Inoculum Production and Inoculation with ectomycorrhizal fungi:-

Host tree must be accompanied by their mycorrhizal associate to survive in areas lacking suitable fungal symbionts. Plantations of exotic pines consistently failed in different parts of the world until suitable ectomycorrhizal fungi had been introduced (Clements, 1941; Hatch, 1936; Briscoe, 1959).

Bakshi (1974) successfully synthesized mycorrhiza of Pinus patula and Sclerodrma geaster only out of many fungi tried. Kannan and Natarajan (1981, 1988) synthesized ectomycorrhizae of Scleroderma citrinum and Amanita muscaria with Pinus patula. Mass production of ectomycorrhizal spawn of Lacaria laccata and Amanita muscaria in sorghum grains has been reported by Raman (1988). Raman and Thiagarajan (1988) reported that 24°C and 28°C were good for spawn growth and 12 hours light moderately good for their growth. Various ectomycorrhizal associates of Pinus Kesiya were tested for their efficiency in colonization, production and growth improvement (Sharma and Mishra, 1989). They reported to have identified certain efficient isolates which could be raised as potential inoculum for P. kesiya afforestation programmes. Kumar (1989) Kumar and Lakhanpal (1991) reported results of artificial inoculation of Pinus gerardiana and Picea smithiana seedlings with mycorrhizal associates isolated from mycorrhiza sphere and
mycorrhizoplane of the natural plants. The survival rate achieved was about 95% and the inoculated seedlings acquired the requisite transplanting height in 1 year instead of 1-1/2 to 2 years. Singh (1992) reported that artificial inoculation of *C. deodara* seedling produce better seedling growth and development. Artificial regeneration with a specific mycorrhizal fungus is therefore doubtlessly beneficial. The inoculated seedling acquire the transplanting height of 25-30 cm in six month time, which almost three times than that achieved with natural inoculum. This suggest a great economic benefit in terms of times, energy and money. Artificial inoculation of *Pinus wallichiana* was reported by Sagar (1993).

Moser (1958a, 1958b) was first to devise a technique for preparation of pure culture inocula of mycorrhizal fungi on peat moss. He (Moser, 1961) utilised several genera of fungi- e.g. *suillus, Amanita, Paxillus, Lactarius, Tricholoma Leucopaxillus* and *Phlemacium* for inoculating *Pinus Cembra* and *P. Sylvestris* and reported differences in stimulation of the growth of pine seedlings inoculated with different species of mycorrhizal fungi. Moser’s technique was further refined by Marx and Bryan (1975). Marx and Bryan (L.C.) used vegetative mycelium in vermiculite-peat moss nutrient medium and basidiospores of *P. tinctorius* to infest fumigated soil and synthesized ectomycorrhiza in *Pinus taeda* seedlings in nursery and concluded that mycelium inoculum of *P. tinctorius* completely colonized roots of loblolly pine seedling during eight month growth period. In North America alone
nearly 2 billion tree seedlings are grown annually in nurseries for artificial regeneration programmes (Marx and Schenck, 1983). Dramatic improvement of tree species with *P. tinctorius* ectomycorrhizae has been reported on acid coal spoils in Kentucky and Virginia (Marx and Artman, 1979), Copper basin of Tennessee (Berry, 1982, 1983), borrow pits (Ruehle, 1980) and stripmined land (Marx, 1975).

Inoculation of containerized seedlings with pure culture is practised in many countries and holds great promise (Dixon et al., 1979; Maronek and Hendrix, 1979, Marx and Barnett, 1974; Marx et al., 1982; Molina, 1980; Pawuk et al., 1980; Ruehle, 1980; and Shaw and Molina, 1980). A mixture of peat moss and Vermiculite is used as an inoculum substrate for *P. tinctorius* and has been successfully inoculated to the containerised seedlings of the genus *Pinus* (Dixon et al., 1980; Marx and Barnett, 1974; Marx et al., 1982; Molina, 1979; Pawuk et al., 1980 and Ruehle, 1980) *Quercus* (Dixon et al. 1980; Maronek and Hendrix, 1979; Ruehle 1980) *Tsuga* (Marx et al., 1982) *Pseudotsuga* (Marx et al., 1982; Molina, 1979). Efficacy of various vegetative inoculum formulation of *P. tinctorius* was compared by Marx et al., (1989) in bare root and container nurseries.

To enhance the productivity of apple, plants requires a balanced nutrition of nitrogen, phosphorus, potassium and other essential minerals. These nutrients are being supplied by the application of fertilizer and minerals in the soil or foliar application. Innovations in different fields of production mananagement like
nutrition, pesticides, mechanisation, soil fumigation, breed of new genotypes, tissues culture techniques for propagation and elimination of systemic pathogens from the planting materials etc. have supplemented the enhancement of apple productivity. In near future severe problems of technological revolution and fertilizer production are imminent because most of these are expensive processes. The production of nitrogen is energy intensive and reservoirs of some fertilizer components. Especially the phosphates are becoming limiting and it is anticipated that in the following decades the gain in horticultural and agricultural productivity will vanish because of limited phosphate for fertilizers and limited energy for other types of fertilizer production. In addition the present fertilization practices are creating many environmental problems such as pollution of ground water and streams etc. So it is desirable that present production practices are changed so as to conserve fertilizer, to prevent waste and save the environmental degradations. But then alternative biofertilizer sources which were renewable and safe have to be found out as substitute for chemical fertilizers. The answer lies in the mycorrhizal fungi whose role in P uptake is well known. Mycorrhiza, a biotic fertilizer (Johnson and Menge, 1981), may provide a alternative to high phosphate applications (Peterson et al., 1984) as a substitute to the present practice of crop production using massive fertilizer applications because there is an inevitable that world shortage of fossil fuels will occur.
The effect of VAM on growth and nutrition of apple plant:-
Mosse (1957) reported that apple seedlings which were inoculated with VA mycorrhizae grow better than controls. Enhanced growth might be due to the very high rate of phosphorus uptake by mycorrhizal roots (Atkinson and White, 1980). Similarly, Plenchette et al., (1981) have reported enhanced growth of apple trees in unsterilized soil under field condition with VA mycorrhiza inoculation. The activity of apple roots can also be beneficially influenced by micro-organism competition. Venter and Thomas (1993) showed that inoculated apple seedlings with VAM fungi resulted in increased dry mass and higher levels of phosphorus and calcium in the roots and phosphorus, calcium, magnesium, copper and zinc in the above ground part of seedling. Catske (1994) observed that phytotoxic micromycetes are thought to cause apple replant disease (ARD). This was suppressed by inoculation of apple seedling with Glomus fasciculatum and G. macrocarpum. After inoculation, growth of apple seedling improved depending on the soil type, VAM fungi species and the ARD degree. It is suggested that the use of VAM fungi can replace chemical treatment of the soil against ARD. Huang et al., (1995) observed that inoculation of apple root stock, seedling with VAM fungi shows resistance to alkaline 8.85 ph soil. Sbrana et al (1995) recorded the mycorrhizal infection in apple enhances both the growth and survival of plant after transplantation. Endomycorrhizal fungi present in greater number in cultivated soils than in non-cultivated soils (Ames and Linderman, 1977).
tion of apple with Glomus sp. was also found to increase shoot and root dry weight (Granger et al., 1983; Hoepfner et al., 1983). In pot culture studies, Sharma and Bhutani (1993) reported significantly superior growth in inoculated apple seedlings with Glomus sp. High chlorophyll content in the leaves of mycorrhizal seedling were also recorded (Sharma, 1994). Increased uptake of P in plant tissues as a result of endomycorrhizal inoculation has been reported in apple (Sharma, 1994; Gnekow and Marschner, 1989; Reich, 1988; Geddeda et al., 1984).

Gnekow and Marschner (1989) observed that VAM inoculation of apple cuttings significantly promoted P uptake at low ($P_1$) and medium ($P_2$) levels of Ca-extractable P. Total dry weight was significantly increased only at the lowest level of available P. However, the fungus, Glomus macrocarpum, appear to have infected apple roots at all P levels. As indicated by elevated Zn concentration in roots ($P_1$, $P_2$) and P levels ($P_3$) and by Cu concentration in roots (all P levels) upon inoculation. Higher concentrations of Zn due to VAM P infection have been found in apple seedlings by Benson and Covey (1976) and in 3 year old peach cutting by Gilmore (1971). Mycorrhizal citrus had higher Cu concentration in shoots and roots (Graham et al., 1986). In annuals the beneficial effect of VAM on Zn and Cu nutrition is well documented (Krishna and Bagyaraj, 1984; Meyer and Linderman, 1986; Pacovsky et al., 1985a; 1985b; Smith and Roncadori, 1986; Sreeramula and Bagyaraj, 1986). With respect to the increased concentrations of Zn and Cu
in roots, it is not clear whether these nutrients are available to the plant as they may be found to fungal polyphosphate granules as has been shown for Ca, Fe and Mn by White and Brown (1979). The stimulating effect of VAM on the uptake of Zn and Cu at high P availability is noteworthy as non-mycorrhizal plants may suffer P-induced Zn and Cu deficiency (Covey et al., 1981; Timmer and Leyden 1978:1980). The enhancement effect of VAM on growth and P uptake (as compared to P fertilization), as well as on Zn and Cu uptake, by apple is almost certainly underestimated by the present results for two reasons: firstly at harvest non-inoculated control plants show level of VAM F infection (due to indigenous fungi) similar to those found in inoculated plants, and secondly on set of VAM F infection was delayed in plants at the lowest P level (20 mgkg⁻¹).

Plenchette et al., (1981) have shown that apple seedlings germinated and grown under greenhouse conditions were inoculated with endomycorrhizal roots originating from a nursery grown Fraxinus prior to out planting into unsterilized field soil. Uninoculated seedlings, in soil either unamended or ammended with 100 kg/ha P as superphosphate, were controls. Shoot length, leaf surface, root volume, stem diameter and dry weight of inoculated plants are all significantly greater than both fertilized and unfertilized controls. No significant difference had been observed in foliar mineral content but the level of N,P,Ca,Cu, and possibly K were higher in the roots of inoculated plants than controls. Although the shoot length of the phosphorus fertilized
plants exceeded that of the unfertilized controls. All the other growth parameters remained unchanged by the addition of P. Intracortical vesicles were absent in the roots of both fertilized and unfertilized control plants where as they were very abundant in the roots of inoculated plants.

Sharma and Bhutani (1992) reported that four species of vesicular-arbuscular mycorrhizal (VAM) fungi were evaluated at five levels of Zn (0, 2.5, 5.0, 7.5 and 10.0 ppm) for root colonization and effects on growth of apple (*Malus domestica* Borkh.) seedlings. Fungi tested were *Glomus fasciculatum* Gerd. & Trappe, *Glomus mosseae* Nicol. & Gerd., *Glomus macrocarpum* Tul. & Tul. and *Glomus versiforme* Berch. & Trappe. The growth of plants in terms of height, trunk diameter, chlorophyll content and total biomass tended to exceed that of non-VAM plants. Fungal species also differed in effects on plant growth. *Glomus fasciculatum* greatly stimulated plant growth as compared to other VAM endophytes. The largest plant were those fertilized with 5.0 ppm Zn and inoculated with *Glomus fasciculatum*. Higher concentration of Zn (7.5 and 10.0 ppm) had adverse effect on growth of mycorrhizal plants.

Mycorrhizal fungal inoculation of apple (*malus domestica* Borkh.) substituted for P application in soil deficient in P of Three mycorrhizal species. *Gigaspora margrita* Becker & Hall was least effective in promoting plant growth, *Glomus fasciculatum* (Thaxter sensu Gerdemann) Gerd & Trappe was the most effective and *Glomus mosseae* (Nicol and Gerd.) Gerd. & Trappe was intermediate.
Combining the three species was no more effective than *G. fasciculatum* alone VA mycorrhiza increased leaf P concentration in apple leaves from 0.04% to 0.19% on parkdale soil which had exchangeable P content of 13 ppm (Geddeda et al., 1984).

Venter and Thomas (1993) reported that the extent to which the roots of plum cuttings and apple seedlings could be inoculated with vesicular-arbuscular mycorrhizal (VAM) fungi under South African conditions, and whether VAM inoculation could result in improved growth and increased plant nutrient levels was investigated. The epidermal tissues of both plum and apple roots were readily penetrated by VAM fungi (colonization was observed in 33 of 36 inoculated plum cuttings and in 29 of the 30 inoculated apple seedlings). Inoculated plants tended to have a greater dry mass and higher levels of phosphorus and calcium in the roots and of phosphorus, calcium, magnesium, copper and zinc in the above ground part of apple seedlings.

Huang et al., (1995) reported that observations were carried out with seedlings of *Malus micromalus*, *M. prunifolia* and *M. sieversii*. They were all sown in pots with VA-mycorrhiza contaminated soil. After 1.-2 years all were transplanted to alkaline soil of pH 8.85. The seedlings treated with VA mycorrhiza grew well and no yellowing leaves were noted. Further observations are required to determine how long this affect lasts.
Soil borne and Aerial diseases: -

Effect of Ectomycorrhiza on White root rot: -

Among the pathogens, *Dematophora necatrix* Hartig causing white root rot of apple is the most destructive pathogen in apple. It has a wide host range affecting 144 plants species (Behdad, 1976). The fungus has been reported to produce macerating enzymes and toxins in the host plant leading to death of roots and maceration of infected tissues (Gupta and Singh, 1985). In India the first report on the occurrence of white root was in the year 1929 by Singh from U.P. hills (Bose and Sindhan, 1976). Later Aggarwal and Sharma (1966) reported it from Himachal Pradesh.

The management of this disease is very difficult because of the location of foci deep in soil on feeder roots where application of fungicide is difficult. The soil itself is a complex structure where the fate of applied chemicals is unknown and has less mobility of effective chemicals in the soil. In addition, high cost of fungicides non-availability of chemicals, chances of development of resistance etc. are also contributing factors to achieve low disease control. Therefore a general strategy comprising of cultural, biological and chemical methods has to be evolved for the management of white root rot. To overcome the revages of the disease, the soil applications of chemical fungicides have been recommended (Gupta, 1977a). However the use of these fungicides have many harmful effects on beneficial microorganisms including mycorrhizae. Reports on the effect of soil
solarization have also been published indicating its use in the management of soil borne plant pathogens (Katan, 1987). Sztejnb-berg et al. (1987) reported that addition of antagonists after solarization gave more encouraging results in controlling Rosellina necatrix. Jain (1961) recommended the addition of lime in acidic soils for control of root rot of apple.

Gupta (1977b) achieved successful control of white root rot by drenching the diseased beds with carbandazim (Bavistin). Aureofungin has also been recommended as soil drench for the management of white root rot by Gupta and Gupta (1992). Mercer and Kirk (1984) reduced the incidence of white root rot of apple with Trichoderma viride. Gupta and Jindal (1990) reported the antagonistic activity of Enterobacter aerogenes against D. necatrix in pot culture and were able to protect the plant upto 45 days of inoculation. Recently Sharma (1993) screened antagonists like Trichoderma viride T.harzianum, Glioclarium virens and Enterobacter aerogenes against D. necatrix in apple and found E. aerogenes to be the best. Though literature is silent about the role of ectomycorrhizae in the management of soil borne diseases of apple but the interaction of soil borne disease in forest trees have shown its potential as bioagent in numerous forest diseases. However, Bharat, (1996) reported that the influence of different VAM fungi namely Glomus mosseae, G. fasiculatum, Acaulospora laevis, Gigaspora gilmorei and Glomus spp. (local apple isolate) was investigated with respect to severity of white root rot caused by D. necatrix. Amongst these, Glomus spp. (local apple
isolate) was found most efficient in colonizing the roots of apple seedlings and reducing the severity of disease. Pre-inoculation of apple seedlings with *Glomus* spp. sixty days prior to *D. necatrix* inoculation lowered the disease severity by 70 percent in comparison to non mycorrhizal pathogen inoculated seedlings.

**Effect of the Ectomycorrhiza on Powdery mildew:**

Plant nutrition has profound impact on resistance/susceptibility of apple trees to various diseases. Except for nitrogen, information on the influence of other macro/micronutrients on plant endurance to diseases is lacking. Fertilizer application with NPK has been reported to increase the susceptibility to apple scab and nonfertilized plants were the least susceptible (Kumar and Gupta, 1986). The role of ectomycorrhiza in the supply of macro/micronutrients to its host has been demonstrated in several forest trees. However specific information on the interaction of mycorrhiza to aerial diseases of apple is lacking. But in general host endurance with balanced nutrition seems to exhibit marked effect with respect to aerial diseases. Therefore, the interaction of mycorrhizal inoculated and un-inoculated apple seedling were also undertaken during present studies.

**Mycorrhizal research in India:**

Research on mycorrhizae in India started in late fifties. The studies on mycorrhizal research have been reviewed recently (Lakhanpal, 1994). Out of the seven mycorrhizal types presently
recognized by Harley and Smith (1983), it is only the ectomycorrhizae and vasicular arbuscular mycorrhizae (VAM), which have been well studied in India, but there is no report available regarding the work of ectomycorrhizal studies on apple. However the morphyology of ectomycorrhiza and the association of various species of VAM with apple in Himachal Pradesh was reported by Thakur and Lakhanpal (1990). While studying the influence of VAM on the growth of apple seedlings, Sharma (1994) observed high chlorophyll content in the leaves of mycorrhizal seedlings than those of non mycorrhizal seedlings. VAM fungi are also Known to enhance the uptake of phosphorus in plants. Evidence of increased uptake of P in plant tissues as a result of endomycorrhizal inoculation has been reported in apple by Sharma (1994).

Jalali and Tharaja (1981) studied the effect of mycorrhizal on the infection of chickpea by wilt pathogen (Fusarium oxysporium f. sp. cicerci) and reported significant reduction in the percentage of wilt infected plants in treatments which were inoculated with mycorrhizal fungus i.e. Glomus sp. Besides this, colonization of roots was also recorded higher with Glomus sp. studies carried out by Jalali and his co-workers (1990) on interaction between. G. mosseae and Macrophomina phaseolina in mungbean revealed that mycorrhizal inoculation significantly restricted the spread of root rot pathogen (M. phaseolina) in host tissues and reduced the incidence of root rot pathogen from 77.90 per cent in pathogen inoculated to 13.30 percent in mycorrhiza.
and mycorrhizal inoculated plants. In pot culture experiments Krishna and Bhagyaraj (1983) reported that the VAM fungus, *G. fasciculatum* provided resistance to peanut plants against *Sclerotium rolfsii*. The number of sclerotia produced by the pathogen, *S. rolfsii* were much less in mycorrhizal roots than non-mycorrhizal roots. Jalali (1978) has reported the control in fusarial with disease of rice with VAM inoculations.