STUDIES ON THE ECOLOGY OF VESICULAR-ARBUSCULAR MYCORRHIZA OF EUPATORIUM RIPARIUM Regel, E. ADENOPHORUM Spreng AND OSBECKIA CRINITA Wall. ex D. Don, THE COMMON WEEDS OF N. E. REGION OF INDIA

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I certify that the thesis entitled "Studies on the Ecology of Vesicular-arbuscular mycorrhiza of Eupatorium riparium Regel, E. adenophorum Spreng and Osbeckia crinita Wall. ex D. Don., the common weeds of N.E. Region of India" submitted by Mr. Nagendra Kumar Verma 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 my 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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GENERAL INTRODUCTION

Amongst a number of weed species common in north-east region of India, Eupatorium adenophorum, Spreng, E. riparium, Regel, and Osbeckia crinita Wall. ex. D. Don, are very frequent. The genus Eupatorium belongs to family Asteraceae and the Osbeckia to Melastomataceae.

E. adenophorum is native of Mexico and was reported from India, first of all from Nilgiris (Fyson, 1915, 1920; Shankarnarayan, 1958). Dutta (1978) reported the wide occurrence of E. adenophorum and its distribution in cultivated fields as well as in open forest areas of N.E. region, between the altitude 1066 m to 2130 m.

E. riparium is native of America, but the literature about its distribution is lacking from India and abroad. MoTooka et al., (1967) reported its occurrence as a weed species from Hawaii while Auld (1970) reported its presence in Australia. Dev (1981) surveyed the distribution of E. riparium and noted that it is very much restricted in distribution. It could be located only around the altitude of 1500 m.

Osbeckia crinita is the indigenous species. Clarke (1879) described two species of Osbeckia, the O. stellata and O. crinita, distributed in Himalaya Terai from Kumaon to Bhutan (alt. 500 ft) and in Khasi hills (alt. 3000-6000 ft). Balakrishnan (1981) merged the O. crinita in O. stellata and reported its wide distribution in Meghalaya. However, in the present work, the name Osbeckia crinita has been retained

because, the merger of the two species is still to get an acceptance.

The success of any plant species introduced in an area depends on a number of ecological factors. Grime (1979) in his ecological classification of plants proposed, the competitors, the stress-tolerators and the ruderals. He also emphasized the importance of mycorrhizal associations particularly, the ectotrophic type, associated with the plant species adapted to nutrient stresses. His conviction seems to hold good for the tree species, as most of such species are reported to have ectomycorrhizal associations and only a few genera of forest trees of economic importance form endomycorrhiza (i.e. Acer, Ulmus, Liquidamber, Fraxinus (Mexal, 1980). However, considering the plant communities as a whole, the ectototrophic mycorrhizal associations are found only in 3 % (Meyer, 1973) or 5 % (Mexal, 1980) plants while the vast majority of the remaining species possess the vesicular-arbuscular type of mycorrhiza (Gerdemann, 1975; Mexal, 1980).

The introduced weed species E. adenophorum is highly aggressive and dominating followed by E. riparium. Although, O. crinita is the indigenous species but compared to the other two, its distribution is very sparce. A third species of Eupatorium, E. odoratum, which is also prevalent at lower altitudes (between 100-950 m.) has been reported to be highly mycotrophic (Graw et al., 1979). It may be that E. adenophorum as well as the E. riparium are also highly mycotrophic and

because of this they are growing luxuriently in the vast area of north east region. The soil of north east region is phosphate deficient and the successful growth of the mycotrophic species in such a condition may be expected. Moreover, the land of this region has been greatly disturbed through the shifting agriculture, resulting into poor soil nutrient status. Therefore, the chances of survival of the plant species entering into the symbiotic relationship with the beneficial microorganisms is more.

The present study was undertaken to evaluate the mycorrhizal status of E. adenophorum, E. riparium and Osbeckia crinata. The study includes the field observations and glass house experiments dealing with the various aspects of vesicular-arbuscular mycorrhiza. The general mycorrhizal status of highly disturbed and relatively less disturbed lands, and the infective potential of the mycorrhizal propagules of the soils have also been evaluated in order to understand the impact of the present agriculture system on the efficient functioning of vesicular-arbuscular mycorrhiza.

REVIEW OF LITERATURE

The work of Reissek "Endophyten der Pflanzenzelle", published in 1847 was a definite description of the association of fungi with the underground parts of plants. Since then, many workers observed such an association and have tried to interpret them. The saprophytic genus Monotropa drew much attention because of its underground parts being completely enclosed within a layer of fungal tissue. Kamienski (1881) interpreted the underground structure in two ways (i) that all the material passing to the plants must pass through this fungal mantle and (ii) that it may be parasitic upon the neighbouring plants.

Working on the same line German botanist Frank (1885) proposed the term "Mycorrhiza" for such a composite organ of fungus and root. He also recognised two types of mycorrhiza, the ectotrophic (having external sheath) and the endotrophic (lacking a sheath). The much faster growth of the mycorrhizal seedling was also hinted by him. Frank (1894) further expressed the view that mycorrhizal plants were capable of absorbing organic nitrogen from a nitrate deficient soil. This "Nitrogen theory" of mycorrhiza was however, not accepted by other workers.

As early as 1905 Galloud reported the presence of vesicular-arbuscular mycorrhiza (VAM) in the roots of many angiosperms and described two types of VA-mycorrhizal infection differing in the extent and location of arbuscles. However, by late 1950's it was realised that the different infection characteristics were caused by the same or a closely related group of fungi.

Peyronel (1923) observed hyphal connections between the fruiting bodies and mycorrhiza in the soil and advocated the inclusion of these endophytes under the genus Endogone. Peyronel et al., (1969) gave three terminology for the common types of mycorrhizas, viz., Ectomycorrhizae, Endomycorrhizae and Ectendomycorrhizae. Since then, these terminology are used world wide.

Asai (1943) raised the plants in sterilized soil as well as in a mixture of sterilized and unsterilized soil and reported the occurrence of mycorrhizal infection in the mixed soil condition. The mycorrhizal plants also showed better growth performance as compared to the non-mycorrhizal plants grown in sterilized soil only.

Garrett (1950) remarked about the evolution of mycorrhiza and wrote, "Evolution of the root inhabiting relationship has culminated in the mycorrhizal association in which a state of true symbiosis has been achieved".

Confirming the earlier observation of Peyronel, Mosse (1953) also observed the fruiting bodies of Endogone attached to extramatrical mycelium of mycorrhizal roots of strawberry. She also established mycorrhizal infection in the strawberry plants and apple cuttings, inoculating the surface sterilized spores and sporocarps and noted the significantly greater growth rate in mycorrhizal plants (Mosse, 1956,1957).

Mosse (1959) further suggested that Endogone spores depend on other soil microorganisms for germination. They also

lack root penetrating ability because under pure aseptic condition, when seedlings were grown in N-deficient inorganic salt medium, the VAM fungus failed to penetrate the plant roots unless assisted by a Pseudomonas sp. (Mosse, 1962).

Mosse and Bowen (1968) gave a broad outline for the identification of different types of Endogonaceous spores based on the nature of cytoplasm, wall structure of spores, presence or absence of attached hyphae, the attachment of hyphae and the colour of spores.

Mosse and Phillips (1971) established mycorrhizal infection in Trifolium parviflorum in a culture medium and recorded that the presence of nitrogen in the media inhibited the infection while the presence of iron accelerated the same. They also found $CaHPO_4$, Ca-phytate, Na-Phytate, Fe-phytate, Phytin, lecithin and DNA as the suitable sources of phosphate for both the symbionts and inositol as suitable source of carbon for Endogones.

Mosse and Hayman (1971) showed the improved growth of the plants pre-inoculated with VAM fungi, transplanted to the pots containing unsterilized soil.

Mosse (1972) further noted that the different strains of mycorrhizal fungi had not the similar effect on plant growth. Among three types of spores used, the E_3 type, stimulated the growth of Paspalum notatum the most, while the laminate spore types proved to be the least effective in all the tests. pH of the soil was also found to be the important factor in determining the effectiveness of different strains. She (1975) indica-

ted that the introduced strains of mycorrhizal fungi were more competent to stimulate the growth of the plants compared to the indigenous ones.

Mosse and Hepper (1975) established the VAM infection in clover root organ culture using a modified white's medium and noted the pH of the medium to be a very critical factor in the establishment of mycorrhizal infection.

Mosse et al., (1976) showed that the VAM fungi could improve the plant growth by utilizing the added rock phosphate from the acid soil while in neutral and alkaline soil it failed to do so. In the same study they also observed that the leguminous plants formed nodules only when they became mycorrhizal and the added phosphate improved the nodulation as well as nitrogen fixation.

Mosse (1977) further exhibited that the introduced strains of VAM fungi were more efficient in utilizing the added rock phosphate compared to indigenous mycorrhizal strains and also the inoculum density in the soil played important role for the response of inoculated endophytes and not the soil phosphate status.

Baylis (1959, '61), obtained increased growth coupled with phosphate uptake in Griselinia. Baylis et al., (1963) also showed that growth of Podocarpus torata and P. dacrydioides was greatly stimulated by mycorrhizal fungi in nutrient deficient soils.

Regarding the role of mycorrhiza in the improvement of

plant growth and phosphorus uptake. Baylis (1967) remarked, "... the phycomycetous endophytes assist uptake of phosphate from soils far below the minimum agricultural standard of fertility. They may occur at higher levels of available phosphate and their presence may be detrimental to the growth of their host, but plants that are growing rapidly because phosphate is readily available and no other factor is limiting, are often free from infections".

Baylis (1970,1972) put forth his idea that the genera deficient in root hairs have greater dependence on mycorrhizas or added phosphates for growth in phosphorous deficient soils than the plants with finely branched root systems and numerous root hairs. Baylis (1975) further added that the primitive angiosperms like members of Magnoliales were more dependent on VA-mycorrhizae for their nutrient uptake even in relatively less fertile soils as they lacked root hairs.

Nicolson (1959,1960) studied the mycorrhizal status of sand dune grasses and reported, variation in the degree of infection in a natural succession. In unstable and building phase with little organic matter, in the stable phase with high organic matter and in the most mature sand dune system, the mycorrhizal infection was low, high and low respectively.

Nicolson (1967) indicated that the cultivated soils harboured more mycorrhizal spores compared to natural and semi-natural communities and that the types of spores and the frequency of their occurrence was subject to the seasonal, annual and local variations.

Nicolson and Johnston (1979) found the Glomus fasciculatus as the only endophyte spreading with the grass roots in a dune system producing spores and sporocarps. They also observed the better growth performance of the mycorrhizal plants in sand dune system but indicated the complexity of interactions between host, endophyte and edaphic factors.

Gerdemann and Nicolson (1963) suggested a technique for the isolation of mycorrhizal spores and sporocarps from the soil by wet-sieving and decanting method.

Gerdemann (1964) also performed growth experiments with maize and tulip plants (1965) and observed the similar results of growth enhancement along with the increased nutrient uptake by mycorrhizal plants over non-mycorrhizal plants.

Gerdemann and Trappe (1974) published a detailed account of endoganaceous spores which proved helpful in the identification and classification of the mycorrhizal spores.

Daft and Nicolson (1966) studied the effect of added phosphates of different solubility in the response of VA-mycorrhizal infection in sand culture and found that the mycorrhizal plants grew better than non-mycorrhizal ones when fertilized with relatively insoluble forms of phosphates. Daft and Nicolson (1969a) also observed that the repeated small doses of soluble phosphate over longer period as well as a larger single dose application in the early growth period, both interfered with the growth of mycorrhizal fungi in maize.

Daft and Nicolson (1969b) showed the effect of different

concentrations of mycorrhizal spores on the initiation and spread of mycorrhizal infection and growth of the plant. Plants inoculated with high number of spores produced more upper leaves and retained more lower leaves whereas plants inoculated with low number of spores, retained more lower leaves than uninfected plants. However, the level of infection was not influenced by the number of spores. They expressed their view in the following words, "As nodulation has been evolved a mechanism for nitrogen fixation, vesicular-arbuscular mycorrhizae may have been evolved as a means for the more efficient extraction of phosphorus from the pedosphere".

Daft and Nicolson (1972) established a quantitative relationship among the size of root system, infection of roots, pigmentation of roots and the ectocarpic chlamydospores production in tomato and maize inoculated with Endogone macrocarpa.

Daft and Okusanya (1973) examined the role of VA-Mycorrhizae in relation to the anatomy and reproductive ability of the plants and found an increase in the amount of vascular tissues as well as flower production in mycorrhizal plants.

The leaves of the plant along with the duration of light has marked effect on the mycorrhizal development and plant growth. Daft and El Giahmi (1978) showed that the defoliation of maize and tomato plants reduced the mycorrhizal incidence and spread whereas the long day favoured the growth response of mycorrhizal plants.

Estimation of the mycorrhizal infections in the roots was a difficult problem, before Phillips and Hayman (1970)

suggested a methodology of clearing the roots by boiling with KOH solution and then staining in cotton blue.

Hayman (1970) studied the spore populations in a wheat field along with the mycorrhizal infection of roots and found them to be influenced by the soil status and the season. He found high number of spores in July which began to decrease after September but remained unchanged from December to June. The effect of formalin treatment of soil was also observed next year with marked decrease in spore numbers. The overall increase in spore number and mycorrhizal infection was recorded in summer.

Hayman (1974) further showed that the host growth was stimulated more with 25000 lux light than with 13000 lux at 23°C and 14-23°C diurnal cycle. No stimulation in growth was recorded at 14°C and 13000 lux light even in low phosphate level of soil. However, the infection rate was high in longer day lengths with high light intensities.

Hayman and Stovold (1979) in a survey of New South Wales soil reported more spores in agricultural soils than in native grassland soils. Hayman and Mosse (1979) also suggested the inoculation of white clover plants with selected VA-mycorrhizal strain for better growth under field conditions.

An increased yield in soyabean was found by Ross and Harper (1970) when an artificial inoculation treatment of VAM fungi was given to a fumigated field plot.

Khan (1972) recorded an overall increase with respect to growth, dry matter production, number of grains per ear along

with the high phosphate uptake in the mycorrhizal maize over non-mycorrhizal ones. He also made a survey of the occurrence of VA-mycorrhizae and recorded the presence of infection and Endogone spores in the rhizosphere of various halophytes, xerophytes and hydrophytes. He found that barring few, almost all the plants were associated with mycorrhizal fungi (Khan, 1974).

Khan (1981) tested the efficiency of Glomus macrocarpus var macrocarpus, G. mosseae, Sclerocystis rubiformis and a E_3 strain of Rothamsted Experimental Station, on the growth of onion in the unsterilized coal washery waste from Coal-cliff collieries and found that the mycorrhizal onions were significantly ($P > 0.05$) better in all the growth parameters than the control. S. rubiformis was the poorest and E_3 type, the most consistent in growth stimulation.

Sanders and Tinker (1971) found that the same source of phosphate is utilized by mycorrhizal as well as non-mycorrhizal plants and that the external mycelium of the endomycorrhizae is responsible for increased uptake of phosphorus. Sanders (1975) further indicated that the high levels of phosphorus within the plants inhibited the infection by VA-mycorrhizal fungi.

Sutton and Barron (1972) described a new technique "floatation-adhesion technique" for the recovery of spores from the soil. They also observed the seasonal change in the spore numbers in the soil. The number of spores showed a decreasing trend depth wise.

Sutton (1973) recorded the three phase pattern of

mycorrhizal development i.e. a lag phase, a phase of extensive development and a phase of constancy in the root of Phaseolus vulgaris.

The extensive mycelial development beyond the root region has the property of aggregating the sand particles. This was shown by Sutton and Sheppard (1976) in Phaseolus vulgarius inoculated with Glomus species, in the sand dunes.

Crush (1973) observed a very interesting dual behaviour of mycorrhizal fungus Glomus tenuis. It depressed the growth rate of grass in the limited conditions of glass house but stimulated the same under field conditions. He (1974) further added that the tropical legumes were much more dependent on mycorrhizas for their growth than the temperate species and correlated this difference with the root hair development. Also, when Trifolium repens and Lolium perenne were grown together, the mycorrhizal association preferentially stimulated the growth of the former i.e. a legume.

Furlan and Fortin (1973) also observed the three phase development pattern of mycorrhizae and added that the temperature alterations affected the duration of these phases. Similarly at different light intensities they recorded the enhanced growth rate of host plant but more pronounced growth was observed under 10 k lux light regime. The light intensity also increased the spore production (Furlan and Fortin, 1977).

Besides other nutrients uptake, the mycorrhizal roots are also capable of absorbing Zn and S, was reported by Gray

and Gerdemann (1973).

Hattingh et al., (1973) indicated that mycelial network of endomycorrhizal fungi enables plants to explore a larger volume of soil beyond the root surface.

Hattingh and Gerdemann (1975) gave a more practical method of establishing the quick and desired mycorrhizal establishment in the seedlings by sowing the seeds pelleted with efficient mycorrhizal inoculum.

Bakshi (1974) along with his co-workers, virtually did a pioneer work in the field of mycorrhizae in India. He studied the ectomycorrhizal as well as the endomycorrhizal associations of Indian trees, and other crops. He also isolated and identified the Endoganaceous spores in the Indian soils and showed their effects on the growth of various plants under different fertilizer treatments.

HO and Trappe (1975) further added to the existing knowledge of mycorrhizae. They observed that the two VA-mycorrhizal fungi, Glomus mosseae and G. macrocarpus had the nitrate reductase system by which they were capable of reducing the nitrate to nitrite.

Powell (1975) examined the roots of Rushes and Sedges and found them non-mycorrhizal. He expressed the view that due to their extensive and finely branched root system they do not need the association of mycorrhizal fungi.

Powell (1976) studied the germination of spores, the developmental stages of the mycelium of the germinated spores

and of those emerging from the root segments, on agar-coated glass slides burried in soil. The spores germinated within 16 days with or without the presence of onion roots. The hyphae developed from the spores formed a fan-lime structure before penetrating the roots while the hyphae emerging from the root segments did it without making any such structure. He presumed, that the different pattern of the infection by the resting spores and the mycorrhizal root-segments were because of their different nutrient supply. He (1976a) found that the introduced VAM strains were more efficient in stimulating the growth of white clover compared to the indigenous ones and concluded that the white clover was highly dependent on infection by mycorrhizal fungi in many hill country soils of New Zealand.

Powell and Daniel (1978) further observed that the mycorrhizal plants could recover the soluble as well as insoluble forms of phosphate more than the non-mycorrhizal plants.

Powell (1979a) used the seeds pelleted with the heavily infested mycorrhizal soils (indigenous) containing Glomus tenuis and Gigospora margarita separately and obtained the more increased growth and dry matter production by the introduced endophytes compared to indigenous ones. He (1979b) also tested the duration value of the superphosphate and five different types of rock phosphates with respect to their efficient recovery by the mycorrhizal fungi. It was observed that the fertilizer value of the superphosphate decreased more rapidly compared to other rock phosphates over successive harvests in all soils. The efficiency of mycorrhizal fungi in the uptake of phosphate

decreased after successive harvest, as the available phosphate levels in soils also depleted.

Powell (1981) reported 27% increase in seed yield of barley with 35% increased phosphorus content of the seed, as a result of inoculation with introduced strains of VA-mycorrhizal strains compared to the indigenous strains.

In an experiment with ^{32}P Rhodes and Gerdemann (1975) found that the phosphate absorption zone of the hyphae of the Glomus fasciculatus was extended upto 7 cm from the root surface of the mycorrhizal roots of onion. Rhodes and Gerdemann (1978) further demonstrated the increased uptake of sulphur by the mycorrhizal roots. They observed that the external mycelium of the mycorrhizal root played important role in the translocation of the nutrients. They also reported that calcium was not translocated as readily as phosphorus.

Cox and Tinker (1976) ruled out the possibility of the digestion of the mycorrhizal fungal hyphae or arbuscles by root cells of the host plant for the phosphate transfer, instead they suggested the transfer of phosphate across the membranes of fungus mycelium, within the host root cells, as the most probable mechanism for this.

El-Giahmi et al., (1976) recommended the use of pre-inoculated plants with some efficient mycorrhizal fungal strains for the better growth performance in soils treated with fungal toxicants.

Gianinazzi-Pearson and Gianinazzi (1976,1978) observed

some qualitative changes in soluble phosphatases in the mycorrhizal onion roots. In the root extracts of onion infected by Glomus mosseae they detected a band of additional soluble alkaline phosphatase specific to VAM infection in Polyacrylamide electrophoretic gel. In continuation, Gianinazzi and Gianinazzi-Pearson (1979) further observed that only immature and little vacuolated terminal arbuscular branches showed significant acid-phosphatase activity while the strong alkaline activities were localized within the vacuoles of mature arbuscles and in the intercellular hyphae.

There are two sources for the propagation of mycorrhizal infection, the resting spores and the already infected roots. Hall (1976) tested their infective ability and found that the mycorrhizal root segments were more effective for the fresh infection of the host roots and subsequently their better growth, in comparison to the resting spores. He also observed that different strains of mycorrhizal fungi had different effect in stimulating the growth of the plants.

Hall (1977) published a descriptive account of the spore morphology and some developmental characteristics of certain specific mycorrhizal fungi. The publication of "A key to the Endogonaceae" by Hall and Fish (1979) is certainly the most upto-date record of the Endogone spores. Almost all the genera and species have been assigned to the appropriate place in the taxonomy of the Endogonaceae.

Hall and Armstrong (1979) indicated that the soil erosion had its adverse impact on the mycorrhizal status of

the soil. The plants grown in the eroded soil respond very less mycorrhizal infection, suggesting the removal of mycorrhizal propagules from the eroded soils.

Kormanik et al., (1977) emphasised the applied value of mycorrhiza and stated that the treatment of nursery beds with adequate endomycorrhizal inoculum may reduce the amount of fertilizer used.

Schoknecht and Hattingh (1976) in a study with X-ray microanalysis found that the cells of mycorrhizal roots of onion, lacking arbuscles, did not contain even the measurable amount of phosphate, whereas the cells with arbuscular structure showed high amount of phosphate.

Smith and Daft (1977,1978) indicated the indirect role of mycorrhizae in symbiotic nitrogen fixation. They demonstrated that at any particular soil-phosphate level, the mycorrhizal infection increased the total P contents, growth, nodulation and nitrogen fixation in Medicago sativa. A delay in the early VAM infection led towards delay in the establishment of bacterial nodulation and finally the nitrogenase activity of the nodules. The nodulation also seemed to be dependent on the P-level of the roots. Smith and Bowen (1979) also suggested to consider the suitable temperature adjustment for the establishment of the dual infection of VAM fungi and nitrogen fixing organism in roots of leguminous plants.

Smith and Smith (1981a) failed to find any toxic effect of soil sterilization on the establishment of mycorrhiza in

Trifolium subterraneum but stated that the growth of Brassica oleracea, which did not form mycorrhiza was better in sterilized soils. The authors also (1981b) compared the effectiveness of the natural and artificial mycorrhizal inoculum and found that the growth responses of plants inoculated artificially were less than those inoculated with natural inoculum and this was related to the delay in the initiation of mycorrhizal infection. They also observed that in artificial inoculation, the location of inoculum in the pot was an important factor in determining the incidence and spread of infection in the host root.

Read et al., (1976) found that all the plant species in the major vegetation types of East Central England were mycorrhizal and the members of Gramineae were particularly more infected. In the limestone grassland the mycorrhizal infection was also observed in the members of Cyperaceae and Juncaceae. Throughout the year they recorded the high infection levels and in the most nutrient stressed situation the infection levels were the highest. It seemed that the means of mycorrhizal propagation was the root to root contact and not the chlamydospores which were always less in number.

In another study Read and Haselwandter (1981) observed that in some Austrian alpine plant communities, the levels of VAM infection were highest in closed herb-rich communities at intermediate altitudes (1900 to 2500 m). In the fertilized hay meadows of lower altitude (1600 m) and in the nival zone, above 3000 m, the endogonaceous infections were light. The fine endophyte Glomus tenuis was the main mycorrhizal fungus coloni-

zing the roots in case of the latter. At different altitudinal range they observed that many plants were infected with a dark septate hyphae which were also present in the roots of the members of Cyperaceae. The ectomycorrhizal associations were also present in some herbaceous species as well as in Salix spp. upto 2500 m.

Abbott and Robson (1977) studied the distribution pattern of mycorrhizal fungi and saw the effect of some endophytes on plant growth. They observed that the non-mycorrhizal plants produced more dry matter in tops at given phosphate concentrations than the mycorrhizal plants at similar phosphate doses.

Abbott and Robson (1978) further added that in unsterilized soil, the inoculation of introduced VA-endophyte did not reduce the infection by the indigenous VA-endophyte. The different endophytes differed in their ability to stimulate the growth and phosphate uptake of subterranean clover but the inoculation with the isolates of Glomus monosporus had more pronounced effect on the growth compared to G. fasciculatus.

The nutrient status of the soil/plant may influence the anatomical behaviour of VA-mycorrhizae to some extent. Abbott and Robson (1979), found that the hyphae in nitrogen deficient plants were slightly wider than those in nitrogen adequate plants. The normal doses of phosphorus had no effect on the branching pattern, the arbuscular-structure, number and other anatomical characters of the hyphae but the addition of phosphate in a dose, more than that required for maximum plant

yield, inhibited the vesicle formation. They concluded, "the anatomy of vesicular-arbuscular mycorrhizas formed by a particular endophyte species, grown under a range of conditions, may not be as variable as has been generally assumed. There is scope for identification of species of endophyte within plant roots. Furthermore, some features of infection morphology could prove to be useful for taxonomic purposes".

Barrow et al., (1977) indicated that the VA-Mycorrhizas had no access to the firmly bound phosphates in soil and that the increased uptake of phosphates by mycorrhizal plants are mostly due to the larger volume of soil explored by the mycorrhizal fungal mycelium.

Becker and Gerdemann (1977) suggested the use of colorimetric method in quantifying the extent of VA-mycorrhizal infections in the roots, instead of other time taking methods. They obtained the significant correlation of percentage of yellow roots by weight, with the yellow colour water extracts, with chitin content and with root/shoot ratio.

Hepper (1977) also used the colorimetric method for the estimation of mycorrhizal infection by digesting the fungal chitin present in the root and subsequently measuring the optical density of the glucosamine, the end product of the chitin digestion.

Hepper (1981) suggested some techniques for the axenic growth of seedlings infected with VA-mycorrhiza using agar, paper or glass as support. These methods were found suitable for

the study of interaction between VAM fungus and the host seedling roots, including spore germination, the hyphal growth and the root penetration mechanism.

Johnson (1977) found most of the plants infected with either Rhizophagus tenuis alone or the mixture of the two and concluded that the R. tenuis was probably the pioneer VA-endophyte in New Zealand forest.

Fitter (1977) hinted that even mycorrhizal infection may be deleterious to a species grown in phosphate deficient soil, if another species growing together has more competitive ability symbiotically.

Azcon et al., (1978) obtained the more pronounced growth in the plants treated with VAM fungi and cell free preparations of Rhizobium, Azotobacter and Phosphobacteria (a pseudomonas), together. The bacterial cultures behaved like pure plant hormone in improving dry weight and infection levels, compared to mycorrhizal control plants.

Mycorrhizal dependency of wheat cultivars was observed by Azcon and Ocampo (1981) but neither mycorrhizal dependency nor mycorrhizal infection levels were found to be directly affected by N.P.K. Ca or Mg concentrations in plant. They further observed that the non-mycorrhizal varieties of wheat cultivars lacked sugar in their root exudation and it was assumed that the VA-mycorrhizal infection results into a decrease in reducing as well as total sugar content of root extracts, which subsequently influences the degree of infection.

Bagyaraj and Menge (1978) observed the synergistic effect of the interaction of Glomus fasciculatus and Azotobacter chroococcum on the growth of tomato. An increase in the population of Bacteria and Actinomycetes was also recorded in the rhizosphere. A reduction in the size and number of root knot galls caused by nematode species, was indicated by Bagyaraj et al., (1979), following the inoculation with mycorrhizae.

Bagyaraj in a subsequent study (1979b) for the first time reported the occurrence of VAM infection in some aquatic plants, from a tropical country, India.

Carling et al., (1978) also found the additive effect of dual infection, i.e. mycorrhizal fungus and nitrogen fixing bacteria on the growth of legumes. Along with the total dry weight, the nodule dry weight also increased which further resulted into increased nitrogenase and nitrate reductase activities of the nodules. The mycorrhizal substitute was the proper dose application of phosphate which had the similar effect as of mycorrhizas.

Cooper and Losel (1978) estimated the lipid content of the mycorrhizal and non-mycorrhizal roots and found that the former contained significantly more total lipid than the latter.

Cooper and Tinker (1981) further demonstrated that the translocation of phosphates in the hyphae occurred normally by protoplasmic streaming but they did not rule out the possibility of a bulk flow of hyphal content, under a water potential gradient.

The reports on the role of VA-mycorrhiza in controlling the diseases or reducing the severity or incidence of the disease is not very encouraging. Davis et al., (1978) found that the mycorrhizal avocado seedlings were more severely attacked by Phytopthora cinnamomi than the non-mycorrhizal controls. No difference in the seedlings of citrus and alfa-alfa was observed, whether it was mycorrhizal or not. Similarly Davis et al., (1979) recorded more severe disease incidence in the Verticillium-wilt of cotton, inoculated with Glomus fasciculatus than the control ones.

Davis and Menge (1981) further added that the association of VAM fungi in citrus, though did not provide any resistance to Phytophthora rot but certainly provided some tolerance to it. In a similar study Davis and Menge (1981) showed that growth response to mycorrhizal infection was reduced to 23 and 58 percent by inoculation with 20 and 50 chlamydospores of Phytophthora parasitica g^{-1} soil, in the sweet orange. Also, 20 chlamydospores of the pathogen had no effect on the percentage infection but 50 chlamydospores reduced it by 72%.

Godse et al., (1978) also reported the enhanced growth of cow-pea when inoculated with the Rhizobium sp. and VA-endophyte together.

The members of Chenopodiaceae and Cruciferae are reported to be free from mycorrhizal infection but Hirell et al., (1978) reported that some plant species of these families may show mycorrhizal infection if grown together with other mycorrhizal plants. Hirell and Gerdemann (1979) also indicated the

possibility of the carbon transfer from one plant to another via VA-mycorrhizal fungal mycelium.

Macdonald and Lewis (1978), on the basis of cytochemical studies noted the occurrence of acid phosphatase, glutamate dehydrogenase, succinate dehydrogenase, glyceraldehyde - 3 - phosphate dehydrogenase, Glucose 6- phosphate dehydrogenase, NADH and NADPH diaphorases in Glomus mosseae and concluded that the fungus possessed an Embden-Meyerhof-Parnas system, a tricarboxylic acid cycle and a hexose monophosphate shunt.

Macdonald (1981) developed a compact autoclavable hydroponic culture system for the production of mycorrhizal infection axenically. The principle behind this was the circulation of liquid at low air pressure with an additional device of the glass fibre air fitters for maintaining the sterility. It had also the provision of sampling the nutrient solution time to time, for analysis and sterility test.

Matare and Hattingh (1978) could not observe any effect of Glomus fasciculatus on the incidence or subsequent development of disease by Phytophthora cinnamomi in the root rot disease of avocado seedlings.

Menge et al., (1978a) further added that the concentration of phosphorus within the plant and not the soil phosphate status, was the regulatory factor in determining the colonization, infection and spore production by VAM fungi.

Menge et al., (1978b) expressed their views that citrus root stocks in general, exhibited the greatest mycorrhizal de-

pendency with the least fertilization.

The method of soil sterilization had its impact on the efficiency of VAM fungi. Menge et al., (1979) suggested to use some selected pesticides to remove the pathogenic organisms from the soil instead of heat treatment which was as harmful to the pathogen as to the mycorrhizal fungi.

Ratnayake (1978) obtained a correlation between the phosphate content of the root and the amount of phosphate added to soil. The root exudation at lower P applications was significantly more in comparison to high P applications. They proposed a hypothesis, "that phosphorus inhibition of mycorrhizal symbiosis was associated with a membrane-mediated decrease in root exudation".

Raven et al., (1978) put forth their opinion that mycorrhizas might have a considerable role in nitrogen nutrition of plants particularly when ammonia is the source of nitrogen.

Sparling and Tinker (1978 a,b,c) studied the mycorrhizal infection in pennine grassland in detail. They observed small seasonal effect in root infection but recorded highest infection in winter and slight variations in root infection at different depths. In case of grasses, the mycorrhizal uptake of P was found to be significant only when the soil was severely depleted, otherwise the fine, much branched root system was sufficient for the same. They also discussed the different responses of various mycorrhizal fungi in relation to phosphorus and lime applications, for the establishment of clover during

improvement of hill grass-land.

Alvarez et al., (1979) reported that the absence of organic layers in the forest of California, favoured the presence of mycorrhiza as well as improved growth of white fir-seedlings.

Atilano and VanGundy (1979) observed no significant difference as regards the number of chlamydospores, among the soils, treated with systemic, non-fumigant and fumigant nematicides. Similarly, there was no difference in fruit yield but fruit loss due to rot disease was reduced in all oxamyl treatments.

The agricultural practice of crop-rotation influences the mycorrhizal status of the field. Black and Tinker (1979) observed that after the rotation of crop, there was a long delay before an appreciable infection percentage developed in the roots, which was followed by a rapid increase and then a constant value. The delay in infection interfered with the crop nutrition and the final yield was found to be negatively related to percentage infection. They further added that other soil factors were not very important in influencing the mycorrhizal infection but a slight correlation between infection percentage, clay content and pH was observed.

Waidyanatha et al., (1979) showed that the growth and nodulation of Pueraria and Stylosanthes and also nitrogenase activity of the former, grown in methyl bromide treated soil were severely retarded unless the plants were infected by VAM

fungi or given a large dose of rock phosphate (500 mg/kg soil).

The VAM infection also helps plant to incorporate more carbon from external sources, compared to non-mycorrhizal plants, was indicated by Losel and Cooper, (1979) in a study with labelled ^{14}C in onion.

Graw et al., (1979) tested the specificity and effectivity of VA-mycorrhiza on 19 host plants and found that Glomus macrocarpus formed efficient mycorrhizal infection with most species, whereas G. gerdemannii could do it only with Eupatorium odoratum. Other mycorrhizal fungi showed the host specificity to lesser degree.

Graw (1979) found that the efficiency of VAM fungi was dependent on pH of the soil which played a significant role in release and absorption of bound phosphates.

Heap and Newman (1980a) demonstrated the mycorrhizal fungal hyphal connections among the roots of the same plant Lolium perenne and also in in between the roots of different plant species L. perenne and Plantago lanceolata, which were growing together in a permanent pasture. The authors (1980b) further, showed that mycorrhizae could increase phosphate transport between different plant species, but failed to explain whether there was a direct transport from one root to another via inter-connecting hyphae or the phosphate first left the donor root, before being taken by the mycorrhizal hyphae.

Mishra and Sharma (1979) studied the distribution of

Endogone species and the mycorrhizal status of the forest trees in the humid forest of Meghalaya. The mycorrhizal association of the important species of ferns of north-east India was reported by Mishra et al., (1980). All the fern species studied possessed the vasicular-arbuscular type of mycorrhizae and the species of Glomus, Gigaspora, Acaulospora and Sclerocystis were isolated from the soils where different fern species were growing. In a recent study Mishra et al., (1981) evaluated the inoculum potential of the mycorrhizal fungi and found that 40 or more endophytes inoculation per plant (in case of maize), produced the highest mycorrhizal infection and growth.

Nemec and O'Bannon (1979) observed that the response of different strains of mycorrhizal fungi was different in the soils treated with different kinds of soil fumigants.

Nemec (1979) further added that the VAM inoculation had good symbiotic effect on the plants irrespective of the diseased or healthy conditions. Nemec (1980) studied the effect of 11 fungicides on the mycorrhizal fungi and found that Captan, Chloroneb, Metalaxyl, NaN_3, and even Captafol had little or no harmful effect on Glomus species.

Nemec and Meredith (1981) detected an increase in the total and free amino acids along with nitrogen in the leaves of non-mycorrhizal plants of citrus.

The increasing rate of air pollution had its detrimental effect on the VA-Mycorrhiza. McCool et al., (1979) recorded a reduction in the height and dry weight of the plants as well as

the reduction in spore number when they exposed the mycorrhizal plants to Ozon and HCl gas.

Miller (1979) found that the disturbed lands were devoid of VAM fungi and also the plants colonizing the disturbed lands were only the non-mycorrhizal.

Moorman and Reeves (1979) and Reeves et al., (1979) screened the plant species of the disturbed lands and found that less than one percent plants in the disturbed lands were mycorrhizal. They also got extemely lower spore population in the disturbed soils and expressed the opinion that the colonization of the non-mycorrhizal plant species on the disturbed lands might effect the successional stages in the natural community ultimately.

Rabatin (1979) noted the highest degree of infection by fine endophyte Glomus tenuis in most phosphate deficient soils and less infection by the same endophytes in the soil rich in available phosphorus, in graminaceous plant species.

The mycorrhizal associations also help host in Zn uptake along with other nutrients. Swaminathan and Verma (1979) observed that the yield responses of crops closely followed the pattern of Zn uptake with exception of potato and even in potato the starch contents of tubers were found to increase commensurately with the level of Zn in leaves. They also indicated that in Zn deficient soils, the mycorrhiza restricts to more easily available fractions of Zn.

The beneficial effect of mycorrhizal associations can

be exploited in a big way by introducing the efficient endophytes in the agricultural fields. Owusu-Bennoah and Mosse (1979) found encouraging stimulated growth of onions, lucern and barley when some selected VAM endophytes were introduced in the field soils.

Weijman and Meuzelaar (1979) examined the biochemical nature of the cell wall of the mycorrhizal spores and found some similarity with the spore structures of the members of zygomycetes.

Antibus et al., (1980) reported that the respiration in the endomycorrhizal roots of Salix nigra and in the ectomycorrhizal roots of S. rotundifolia was only partially sensitive to cyanide treatments.

Allen and Allen (1980) found five of the seven annuals, colonizing the stripemine reclaimed sites in Wyoming as non-mycorrhizal. The spore population and the mycorrhizal infections were also low at these sites, compared to the native prairie levels.

A significantly increased cytokinin concentrations in the leaves and root of mycorrhizal plants was also recorded by Allen et al., (1980). Allen et al., (1981a) further studied the effect of various combinations of phosphate sources on the establishment of VAM fungi in Bouteloua gracilis and found Ca-phytate as the more suitable P-source for the more beneficial symbiotic association. Besides improved growth and increased P content, they also noted the high chlorophyll concen-

trations and more alkaline phosphatase activities in the mycorrhizal plants than in the non-mycorrhizal plants. In another study, Allen (1981b) found the enhanced water and nutrient uptake and also photosynthetic rate in mycorrhizal B. gracilis.

Chambers et al., (1980a) showed the inhibitory effect of the combined nitrogen treatment in the form of $NaNO_3$ and $(NH_4)_2SO_4$ on the development of vasicular-arbuscular mycorrhiza, the nodulation and the nitrogenase activity in Trifolium subterraneum. The overall growth also decreased by the combined nitrogen treatments. Chambers et al., (1980b) further indicated that the mycorrhizal infection was also reduced by two nitrification inhibitors, namely 2-chloro-6 (trichloromethyl) pyridine and 2-trichloromethyl pyridine.

In a recent publication Giovannetti and Mosse (1980) discussed in detail about the merits and demerits of the various evaluation techniques used for the mycorrhizal studies.

Haselwandter and Read (1980) studied the fungal association in dominant and sub-dominant plants of high-alpine vegetation system and found Glomus tenuis as the most common endophyte associated with the most of the plant species. They also found the constant association of Rhizoctonia species with a non-mycorrhizal plant Carex and hinted towards the possibility of Rhizoctonia sp. behaving like a mycorrhizal association. About the reduced infection at higher altitude they remarked "The lower infection found at higher altitudes may be a result of reduced host photosynthesis and hence lower assimilate supply to the mycobiont".

Kellam and Schenck (1980) studied interaction between VAM fungus Glomus macrocarpus and a root knot nematode Meloidogyne incognita in soybean and found that the plants infected with both the organisms had significantly fewer galls per g root, greater root weights and higher yields than those infected with nematode only. They also observed that the presence of mycorrhizal fungi reduced the number of galls formed by the nematode and the presence of the nematode affected the mycorrhizal development only in the immediate area of the gall. However, the nematodes, had little effect on the mean percentage infection and the chlamydospore production by the VAM fungus.

Kelley (1980) established VAM association in Nyssa sylvatica under flooded condition and noted the most mycorrhizal association near main root region and suggested that under flooded conditions, the internal oxygen transport might be a limiting factor to mycorrhizal development in the distal parts of root.

The reduction in mycorrhizal infection by the Boron deficiency in soil was reported by Lambert et al., (1980). They (1980b) further indicated that the indigenous mycorrhizal propagules were more efficient for the beneficial effects on plants, because the non-indigenous strains could not adapt to the changed edaphic factors.

Levy and Krikun (1980) published a paper on the water relations of Citrus jambhiri influenced by VA-mycorrhiza and stated that the mycorrhiza could help plants to recover from

water stress by regulating stomatal movements.

Ocampo et al., (1980) indicated that the barriers to VAM infection in "non-hosts" might be due to some structural characteristics of root cortex or epidermis and not because of root exudates. When the "non-hosts" were grown with same host species, the former could get slight infection by VAM fungus.

Ocampo and Hayman (1981) found that the amount of VAM infection in a host plant was not depressed in soil previously cropped with a "non-host" plant, even when the roots of preceding "non-host" plant were retained intact in the soil. Contrary to the expectation, the presence of "non-hosts" stimulated the early establishment of VAM in the host crops. They also observed the vesicular-arbuscular hyphae growing in the moribund roots of "non-hosts".

Ojala and Jarrell (1980) developed a hydroponic-sand culture system for mycorrhizal studies and obtained favourable results with regards to yield and other growth parameters. The advantage of the system is the recycling of the nutrient solutions several times a day.

Pairunan et al., (1980) examined the effect of soluble and insoluble sources of phosphorus on the efficiency of VA-Mycorrhiza. They found no difference in the growth and dry matter production by mycorrhizal or non-mycorrhizal white clover at any source of phosphorus given. At certain concentrations of phosphorus given, in tops, the non-mycorrhizal plants produced more dry matter than the mycorrhizal plants supplied with super-

phosphate. However, the mycorrhizal induced growth response was more obvious at intermediate doses of phosphate application. They also reported more uptake of Zn and less Ca by the mycorrhizal plants.

Rose (1980) reported VAM associations in some actinomycetous nodulated nitrogen fixing plant species. Rose and Youngberg (1981) also studied the effect of mycorrhiza on these plant species and found the increase in growth as well as the P and N contents of the plants having this tripartite association.

Ross (1980) advocated the existence of the "mycosphere" surrounding the mycelium of VAM fungus which enabled the roots to absorb the metabolites produced by other organisms in the region. He further, stated that these accumulated metabolites within the fungus and/or root played important role in inhibiting or reducing the sporulation of mycorrhizal fungus within and outside the root.

Schenck and Kinloch (1980) studied the early colonization of roots and extramatrical production of spores in 6 crops grown as monoculture for 7 years in a newly cleared site and got variable response of different types of mycorrhiza on different crops. They concluded that such variable responses were little affected by other factors except the specific host characteristics which seemed to be more important.

About the mycorrhizal role in disease control, Schenck (1981) pointed out "Past and current research indicates that mycorrhizal fungi can deter or significantly reduce the effects

of some pathogens on the host. Most of the evidences, however, are from laboratory green house, or microplot studies. Little work has been done in the field, and no deliberate effort has been made in commercial agriculture to control root disease with mycorrhizae. In my opinion, preliminary results of green house studies look promising and justify further investigations".

Warner and Mosse (1980) on the basis of a short experiment reported that the VAM fungi could spread independently in soil and seemed to play some saprophytic role in soil system.

Biermann and Linderman (1981) proposed a different method of evaluating the mycorrhizal status of plant in which they estimated the percentage of length of the root segments containing VAM fungal structure, instead of determining the percentage infection of roots. They got the confidence limits within 10% of the mean when 7 samples, each with 25 randomly selected 0.5 to 1.0 cm. root segments were examined.

Bradley et al., (1981) reported that in Calluna vulgaris the mycorrhizal association provided the resistance to heavy metals toxicity and also it led to the significant reduction of heavy metal content of the shoot.

Clarke and Mosse (1981) obtained better yield, when Barley crop was inoculated with VA-endophyte in field. They also found that without any addition of phosphate in soil, only mycorrhizal infection doubled the production of ears in terms of fresh weight but when phosphate was added it increased the ear production more than inoculation.

Daniels and Menge (1981) hinted the commercial production of VAM fungus Glomus epigeous. More sporocarps were produced by the endophyte in association with Sudan grass and also better growth response was recorded in a number of crops when inoculated with G. epigeous. The high spore producing capacity, efficiency on a wide range of host plants, ease of storage and less loss of germinability of this VAM strain was considered of great potential value for commercial production and effective exploitation.

Gould and Liberta (1981) found that the inoculum potential of the mycorrhizal spores decreased due to soil storage and this decrease was proportionate to the increase in storage time.

Besides growth and high phosphorus content, a very much higher hydraulic conductivity of the root system was observed in mycorrhizal plants compared to non-mycorrhizal ones by Hardie and Leyton (1981). They also observed that under water stress conditions the mycorrhizal plants developed higher leaf area than the non-mycorrhizal plants and that they could survive at such soil water potential which were below than that to be tolerated by the non-mycorrhizal plants.

Krishna et al., (1981) showed a number of anatomical and histochemical differentiation in the mycorrhizal and non-mycorrhizal Eleusin coracana. They noticed well marked increase in the thickness of leaves, size of mid-rib vein, major, minor and last veins, the motor cells, mesophyll cells and number of plastids in the leaves of mycorrhizal plants. Also, the leaves

of the mycorrhizal plants contained higher amounts of insoluble polysaccharides and insoluble proteins than the leaves of non-mycorrhizal plants.

Manjunath and Bagyaraj (1981) studied the effect of different components of VAM inoculum on the growth of onion. The infected root segments and the extramatrical chlamydospores, both stimulated the growth but the former was more efficient compared to the latter. However, the use of only associated microorganisms of the roots or the sterilized infected segments as inoculum, failed to enhance the growth at all.

Manjunath et al., (1981) studied the effect of inoculation of Glomus fasciculatus, Beijerinckia mobilis and Aspergillus niger either singly or in combination, on onion plants. Except A. niger, they found better plant growth, when inoculated singly the remaining two inoculum. They also found that the inoculation with B. mobilis stimulated the spore production by VA-Mycorrhiza. In general, the combined inoculation treatment was found to be much beneficial than single.

Rich and Schenck (1981) made a comparative study of the plant parasitic nematode and mycorrhizal chlamydospores and obtained a positive correlation between the two. They recorded more spore and nematode in first 15 cm. soil depth but the depth-wise variation was found to be influenced by the individual plant species.

Saif (1981) found that the oxygen concentration in the soil atmosphere greatly influenced the growth and mineral uptake

of the host Eupatorium odoratum infected with Glomus macrocarpus. At all levels of oxygen concentration, the mycorrhizal plants produced more shoot and root dry weights than non-mycorrhizal ones, except at 0% level. He also found a positive correlation between nutrient uptake and the increasing O_2 concentrations in mycorrhizal plants. Phosphate content of mycorrhizal and non-mycorrhizal plants differed significantly but there was no relationship with O_2 concentrations.

Schroder et al., (1981) suggested the transplantation of the fast spreading mycorrhizal inoculated grass species in the field, for the extensive spread and establishment of VA-mycorrhiza in fumigated mycorrhiza free soils.

John (1981) described a simple method for synthesizing the pure two membrane VAM infections, using very simple culture media.

On the basis of ultrastructural studies and the localization of the ATPase activities on arbuscles Marx et al., (1982) proved the earlier hypothesis right that the nutrient exchange in VA-mycorrhizas occur across the living host fungus interface and also the phosphate transfer from fungus to host is facilitated by the active transport mechanism in the finer branches of arbuscles.

Jensen (1982) found that Glomus constrictus and the two isolates of G.fasciculatus enhanced the growth rate of Barley along with the increased uptake of P, Cu and Zn whereas Gigaspora margarita proved very poor to do so and the effect was not more than the non-mycorrhizal control.

STUDY AREA AND CLIMATE

The present study was carried out at Shillong which is situated at 35°34'N, 91°56'E. The altitude varies from 1250 to 1960 m. Physiographically the entire area is hilly covered with pine forests (Pinus kesiya). The soil is red laterite under red loam or brown loam soil type. The sand content of the soil is upto 90 % at some places and it is acidic in reaction with pH ranging from 4.9 to 6.7. The soil is rich in nitrogen content in the form of organic matter (above 7.0-7.5%). However, the amount of phosphorus is very low ranging between 20 Kg/acre to 50 Kg/acre.

The climate of Shillong is cool with winter temperature goind down to 4-5°C in the month of January. The lower temperature of winter results into frost which can be seen sometimes early in the morning during December and January. The maximum temperature goes upto 25°C in the month of April. The average maximum temperature is 20.72°C and minimum temperature 12.77°C. The rainfall is spread over all the months except November, December and January when it is either nil or very less. The average annual rainfall is 173.53 mm. Similarly the average humidity is very high ranging between 71.38 to 84.21 in a diurnal cycle.

The typical summer season is not found at Shillong. However, based on the meteorological conditions the year can be divided into following seasons.

Winter season:- Winter season starts at the end of October and

continues upto middle of February. The average lower temperature during winter is 7.9°C and maximum temperature 16.61°C. The rainfall is very low.

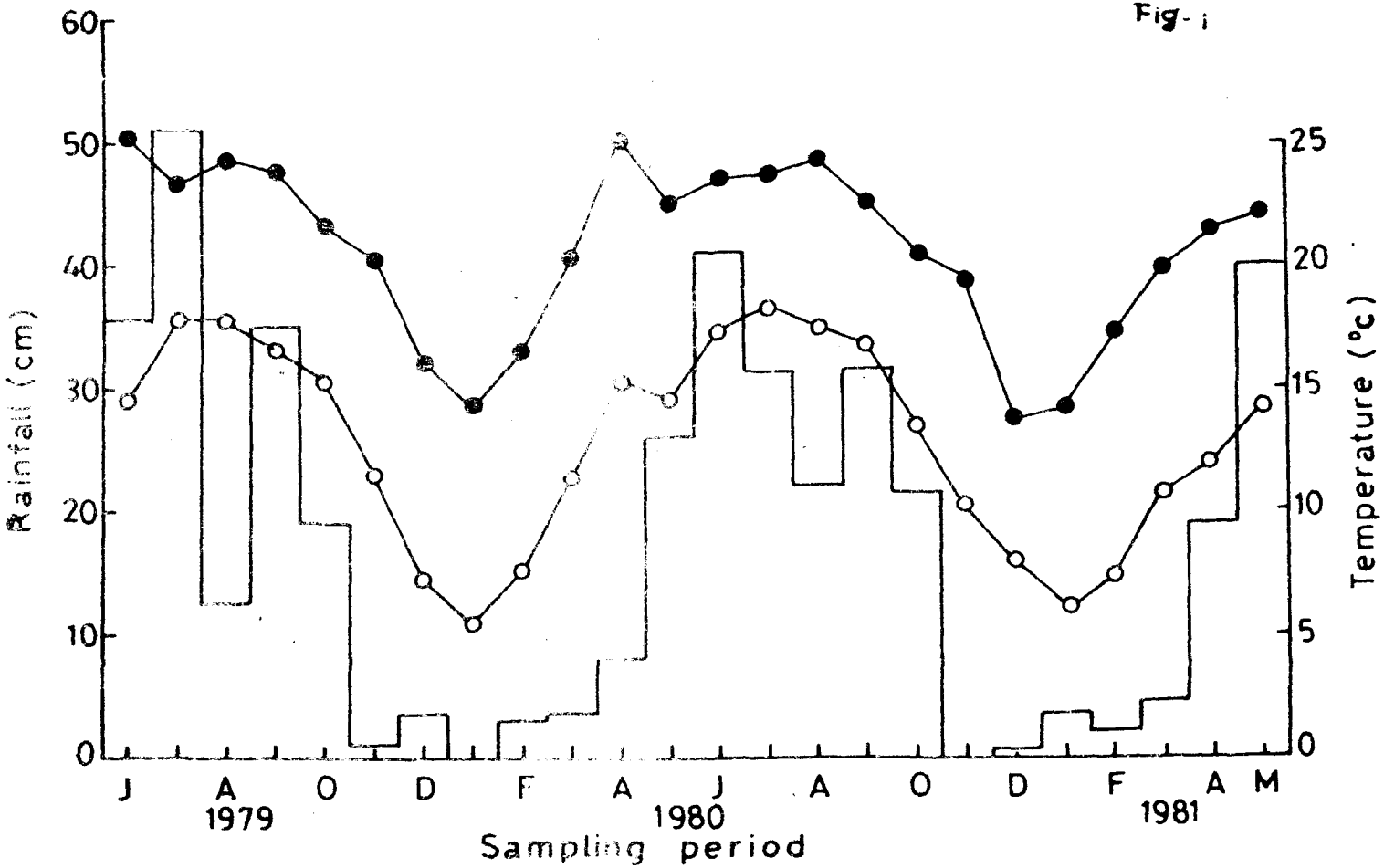
Spring season:- The period from the middle of February upto middle of April covers the spring season which experiences very high wind velocity with less humidity and moderate temperature.

Rainy season:- The rainy season extends from the middle of April to the middle of October. However, the early period of the rainy season is a bit warm representing summer, while the later period of the season is comparatively cool. Due to high rainfall the humidity is also very high during rainy season.

The data of the temperature and rainfall, during the study period (1979-80 and 1980-81) has been presented in Fig. 1.

Fig. 1 Data of the temperature and rainfall during the period of 1979-81. Histograms, rainfall; ●——●, mean daily maximum temperature; O——O, mean daily minimum temperature.

Fig- i


60
50
40
30
20
10
0
Rainfall (cm)
25
20
15
10
5
0
Temperature (°c)
J
A
O
D
F
A
J
A
O
D
F
A
M
1979
1980
1981
Sampling period

CHAPTER I

Studies on the vesicular-arbuscular mycorrhizae of Eupatorium adenophorum Spreng, E. riparium Regel, and Osbeckia crinita Wall, ex. Don. under natural conditions.

Introduction

Eupatorium adenophorum, E. riparium and Osbeckia crinita are the three common weed species of north-east India. They occupy a vast area in cultivated land as well as in forest. The wide occurrence of these weed species have the wide spread root system in soil and therefore, the rhizosphere activity of these weed species may have marked influence on the microbial activity, the mycorrhizal activity and subsequently the general soil fertility of the region.

The rhizosphere of the plant species is the site of greater microbial activity and also harbour the higher microbial population than the adjacent soil (Katznelson et al.,1948; Clark, 1949; Starkey, 1958; Rovira, 1965 and Timonin, 1965). The seasonal changes, the soil fertility and the age of the plants, affect the mycorrhizal infection and the extramatrical spore population (Hayman, 1970; Mosse, 1973a). The sources of the mycorrhizal propagation under natural condition are the infected root systems, the endogone spores produced by them and the mycorrhizal hyphae spread in soil. The vesicular-arbuscular mycorrhiza is least host-specific (Mosse, 1973a; Gerdemann, 1975) and thus the wide spread root-system of any plant species may enrich the general mycorrhizal status of the

field soils. The higher mycorrhizal potential of the soil can support the better growth of the crops also.

The present work was undertaken to evaluate the mycorrhizal activity within the root and also in the rhizosphere of the three weed species in relation to the changes in the soil nutrient concentration. In addition to this, the fungal flora of the rhizosphere has also been assessed.

Material and Methods

The pure stands of Eupatorium adenophorum, E. riparium and Osbeckia crinita, were selected for the study. Several small seedlings of E. adenophorum and E. riparium were tagged just after their emergence in the month of March/April in nature. But in case of Osbeckia crinita, no seedlings were found to be emerging from the seeds and all the sprouts emerging in the month of March/April were traced to be connected with the tuberous roots lying beneath the soil surface. So, the roots and rhizosphere soil, in case of O. crinita were collected only from the new roots coming from the young sprouts.

Collection of Samples- The area of 20 m x 20 m was marked in the pure stands of the three weed species. From this area the soil with intact root system upto 10 cm. depth was collected from 6 different spots and brought to laboratory in sterilized polythene bags. The sterilized polythene bags were used only for the year 1979-80, when the rhizosphere fungal flora was also estimated.

Further, in the laboratory, the roots of all the three weed species were separated from the soil for the inoculation of the rhizosphere fungal flora and for the mycorrhizal infection assessment, separately. The separated roots of the replicates were mixed to form a composite sample of root and similarly the soils were mixed for the composite sample of soil. The pH and the moisture content of the rhizosphere soil were assessed immediately. The inoculation for the rhizosphere fungal flora was also done within 4 hours of the collection. The mycorrhizal infection was assessed either same day or otherwise, the roots were preserved in F.A.A.

Assessment of rhizosphere fungal flora- The fine roots (mostly tertiary) which are mostly infested with the vesicular-arbuscular mycorrhiza, were gently tapped in order to remove the extra soil particles from the root surface and the roots with closely adhered soil particles were transferred to the conical flasks containing 100 ml. sterilized distilled water. These conical flasks were shaken on the mechanical shaker for 15 minutes and finally 0.5 ml of the suspension was inoculated in each 5 petriplates containing Rose-Bengal-Agar medium (Martin, 1950). The inoculated plates were incubated for 6-7 days at 25^{o}C in B.O.D. incubator. The total fungal colonies which appeared after incubation were counted and the fungal species were identified. The weight of the rhizosphere soil was determined after removing the root from the conical flasks and evaporating the water from the soil suspension first on the hot plate and then in a hot air oven at 105^{o}C for 18 hours. After drying, the conical

flasks were cooled and weighed, and the weight of the conical flask was deducted from this weight, which finally gave the weight of the rhizosphere soil suspended in the water.

The total fungal population was calculated as follows:

Total no. of fungi/g soil

$$= \frac{\text{No. of fungal colony X dilution factor}}{\text{Weight of rhizosphere soil (g)}} \text{ X } 100$$

Determination of mycorrhizal infection and isolation of Endogone spores- The root was cut into approximately 1 cm. segments and boiled in 10% KOH for 90 minutes, washed with water and slightly acidified with 5% acetic acid. The staining was done in cotton blue and the stained segments were observed under microscope for the estimation of infection. The method was followed according to Phillips and Hayman (1970). The percentage infection was calculated as follows:

$$\% \text{ infection} = \frac{\text{No. of infected segments}}{\text{Total segments observed}} \text{ X } 100$$

The Endogone spores were isolated by wet-sieving and decanting technique (Gerdemann and Nicolson, 1963). 10 g soil was stirred in 500 ml water, in a beaker, allowed to stand for one minute and decanted to pass through the sieves of the size 150 μ, 90 μ and 50 μ. The process was repeated thrice and the spores retained on the sieves were washed thoroughly in tap water. Further, all spores were collected together and filtered on the Whatman No. 1 filter paper. The spores were counted under simple stereomicroscope.

Soil analysis:-

pH and moisture content determination- 10 g of soil was diluted in 50 ml distilled water and stirred for 15 minutes on mechanical shaker. The pH was read in an electric digital pH meter. For the determination of moisture content, 10 g soil was dried in a hot air oven at 105°C for 24 hours and weighed. The percentage moisture content was calculated as follows:

$$\% \text{ moisture content} = \frac{\text{Loss in weight on drying (g)}}{\text{Initial sample weight}} \times 100$$

Organic matter determination- 1 g sieved soil (through 0.2 mm. sieve) was taken in a 500 ml conical clask in which 10 ml $K_2Cr_2O_7$ (1N) and 20 ml Conc. H_2SO_4 were added and left for 30 minutes. After that, 200 ml distilled water and 10 ml ortho-phosphoric acid (85%) were added and finally titrated with $FeSO_4$ (1N) using diphenyl amine as indicator. The percentage organic matter was calculated according to the formula given below:

$$\% \text{ organic matter} = \frac{V_1 - V_2}{W} \times 0.003 \times 100 \times 1.724$$

Where, V_1 = Volume of $K_2Cr_2O_7$

V_2 = Volume of $FeSO_4$

W = Weight of soil (g).

Total nitrogen determination- 10 g sieved soil was transferred in a 300 ml digestion flask and moistened with 25 ml of distilled water. After about 20 mnts, 20 g mixed catalyst (20 g copper sulphate + 3 g mercuric oxide + 1 g selenium dioxide) and 35 ml

of Conc. H_2SO_4 were added. The digestion was done first by low heating and afterwards at high temperature, in digestion units. The digestion was completed in about 2 hours, when the whole content was diluted with distilled water in a 800 ml flask and distilled in presence of 40% sodium hydroxide. The released ammonia was absorbed in 4% boric acid and titrated with $N/_{14}$ HCl. The percentage nitrogen was calculated as follows:

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

Where, T = Sample titration

B = Blank titration

N = Normality of Acid

S = Sample weight (g)

Determination of available phosphorus:- The available phosphorus was first extracted in Ammonium fluoride extraction solution. The extraction solution was prepared by mixing 15 ml NH_4F solution (37 g/1000 ml) with 25 ml HCl (0.5 N) and 460 ml distilled water. 4 g sieved soil was taken in 100 ml conical flask in which 14 ml extraction solution was added and stirred for 15 minutes on mechanical shaker. The extract was filtered through Whatman No. 44 filter paper. 2 ml of this aliquate was diluted with 5 ml water and further added with 1 ml ammonium molybdate and 2 ml stanus chloride to develop the blue colour and reading the optical density. The O.D. was read at 660 nm. and converted into known units through the standard graph. The available phosphorus (ppm) was calculated as follows:

$$\text{Available P (ppm)} = \frac{\text{ppm of P in solution X combined volume}}{\text{aliquate (ml) X sample Wt (g)}}$$

Determination of exchangeable potassium- The potassium was extracted in Ammonium acetate solution (pH 7), which was prepared by mixing 575 ml of glacial acetic acid with 600 ml of ammonia solution and diluted to 10 liters with distilled water. The pH was adjusted to 7 $\pm$ 0.05 with the help of acetic acid or ammonia solution. 10 g sieved soil was stirred with 250 ml of extraction solution for one hour and filtered through Whatman No. 44. The exchangeable potassium was read in a photo-flame meter and converted into known unit through standard graph. The calculation was done according to the formula:

$$\text{exchangeable K (mg/g)} = \frac{\text{C (ppm) from graph X solution volume}}{10^3 \text{ X sample wt (g)}}$$

All the methods of soil analyses were followed according to Jackson (1967) and Allen (1974).

Results

Mycorrhizal infection:

The mycorrhizal infection in E. adenophorum ranged between 50 to 80 percent throughout the year except in winter months when it was very high. The infection level was comparatively higher in 1980-81 but the pattern of infection remained

similar during both the years (Fig. 1a). The analysis of variance showed that the mycorrhizal infection was negatively correlated with most of the data with respect to soil properties as well as climatic factors (Table 1.4), but the significant relationship was noticed only with maximum and minimum temperature.

Like E. adenophorum the higher infection percentage was also recorded in E. riparium during winter months and the maximum infection percentage was observed in January (1979-80) and December (1980-81). A depression in infection occurred in March (1979-80), April (1980-81) and in September (in both the years) (Fig. 1b). The mycorrhizal infection did not follow the similar pattern during both the years. In 1979-80 it was found to be positively correlated with pH, nitrogen (significant at 5% level) and potassium but negatively correlated with others. The negative correlation was found to be significant with maximum temperature (5% level) and minimum temperature (1% level) while in 1980-81 it was not significant statistically (Table 1.5).

The infection pattern in Osbeckia crinita was quite different from the other two species. The infection showed the increasing trend from March/April i.e. from the beginning to December i.e. the end of the life cycle and almost similar pattern was observed in both the years (Fig. 1.2). The analysis of variance showed that the mycorrhizal infection was negatively correlated with the climatic data and the soil properties

except with organic matter and total nitrogen in 1979-80 and with available phosphorus in both the years (Table 1.6).

The observation of the roots for the mycorrhizal infection revealed that in a 12 month cycle sometimes dual and/or triple mycorrhizal infection (distinguished morphologically) developed in the root cortex. The external vesicles (characteristics of Gigaspora sp.), the fine endophyte (characteristics of Glomus tenuis) and the coarse endophyte (formed by more than one mycorrhizal strain) were observed either singly or in associations sometimes. When the percentage infection by G. tenuis was estimated in the year 1980-81, in case of E. adenophorum and E. riparium it was found to be negatively correlated (significant at 5% level) with the average temperature and particularly with lower temperature of winter months (Fig. 1.3).

The higher spore population was observed in the rhizosphere of E. adenophorum throughout the year in 1979-80 and 1980-81. Two peaks of maximum spore numbers, one in August and another in December were observed during the year 1979-80 but in the year 1980-81 no marked fluctuation in spore population could be observed (Fig. 1.1-a). The relationship of spore population was found to be positive with moisture content, the organic matter and the total nitrogen, but negative with the others. The significant correlation was obtained only between spore population organic matter and nitrogen in 1980-81 (Table 1.4).

The spore population in the rhizosphere of *E.riparium* was comparatively less in 1980-81 than the previous year. The gradual increase in spore population was observed from June to November, which further declined gradually in 1979-80, but no trend could be observed in 1980-81 (Fig. 1.1.b). In the year 1979-80 a significant ($P > 0.05$) positive correlation was found between spore population, pH and organic matter while the relationship with maximum temperature was negative (significant at 5% level). However, no correlation was found to be significant during 1980-81 (Table 1.5).

In case of *Osbeckia crinita* the spore population showed only little fluctuation upto August which decreased in September, but later on a different trend was followed in the subsequent year. During the period 1980-81, the spore population increased to maximum peak in October and declined subsequently. In 1979-80, however, it maintained a continuous increase upto December (Fig. 1.2). The negative relationship of spore population with maximum temperature, minimum temperature and the rainfall was found to be very significant ($P > 0.01$) in 1979-80 while in 1980-81 the significant ($P > 0.05$) relationship could be obtained only with nitrogen (Table 1.6).

Based on morphological characters three mycorrhizal fungi could be identified viz., *Gigaspora* sp., *Glomus* sp. and the species of *Sclerocystis*. The *Gigaspora* sp. were found to be very less (never more than 8 in 10 g soil) while the *Sclerocystis* sp. could be seen only rarely. The majority of

the Endogone spores probably belonged to more than one species of Glomus.

Rhizosphere fungi:

The total fungal population in the rhizosphere of E. adenophorum and E. riparium exhibited almost similar pattern throughout the year (Fig. 1.4). It was less and remained unchanged from March to June which began to increase afterwards. An abrupt decrease in fungal population was noticed in September which again increased sharply but afterwards it maintained a decreasing trend. In case of Osbeckia crinita very less number of total fungi was recorded and also the trend was different from the other two species. It remained unchanged upto June but showed the continuous increase, subsequently, reaching maximum in December (Fig. 1.4). The fungal population in the rhizosphere of O. crinita showed significant positive relationship with Endogone spores ($P > 0.05$) and infection percentage ($P > 0.01$) and significant negative relationship with maximum temperature ($P > 0.01$) and minimum temperature ($P > 0.05$). The relationship of the rhizosphere fungi in case of E. adenophorum and E. riparium was not found to be significantly correlated with any of the factors. The most common fungi present in the rhizosphere were, yeasts, Trichoderma viride, Penicillium sp., Phoma humicula along with the Sterile white mycelium colonies which were frequently isolated (Table 1.7).

Fig. 1.1 The percentage VA-mycorrhizal infection and *Endogone* spore population in two year cycle. a, *E. adenophorum*; b, *E. riparium*.

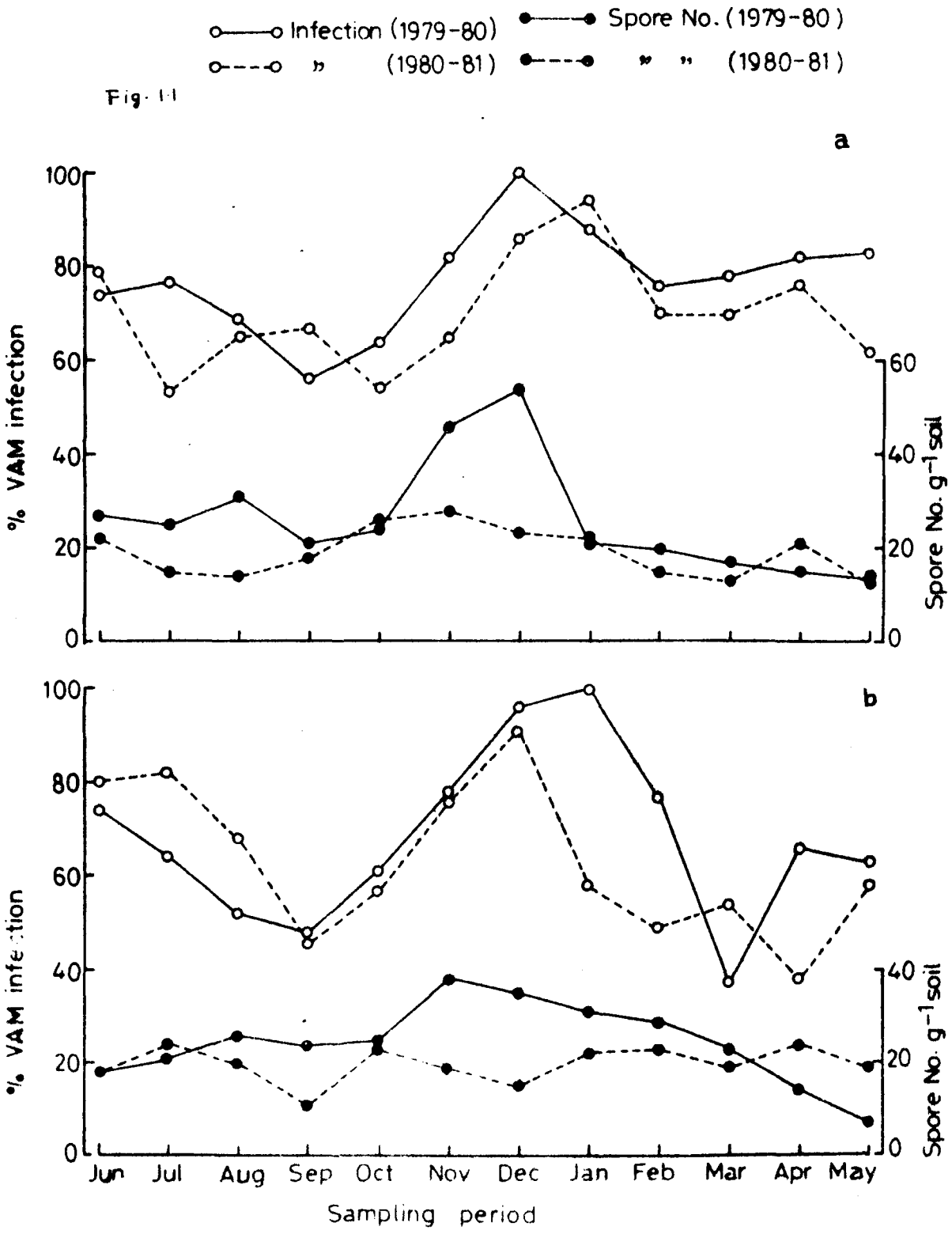

Infection (1979-80)
Spore No. (1979-80)
» (1980-81)
» » (1980-81)
a
b
% VAM infection
Spore No. g⁻¹ soil
0
20
40
60
80
100
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Sampling period

Fig. 1.1

Fig. 1.2 The percentage VA-mycorrhizal infection and *Endogone* spore population in two year cycle of *Osbeckia crinita*.

o----o Infection (1979-80) ●----● Spore (1979-80)
o——o " (1980-81) ●——● " (1980-81)

Fig-12

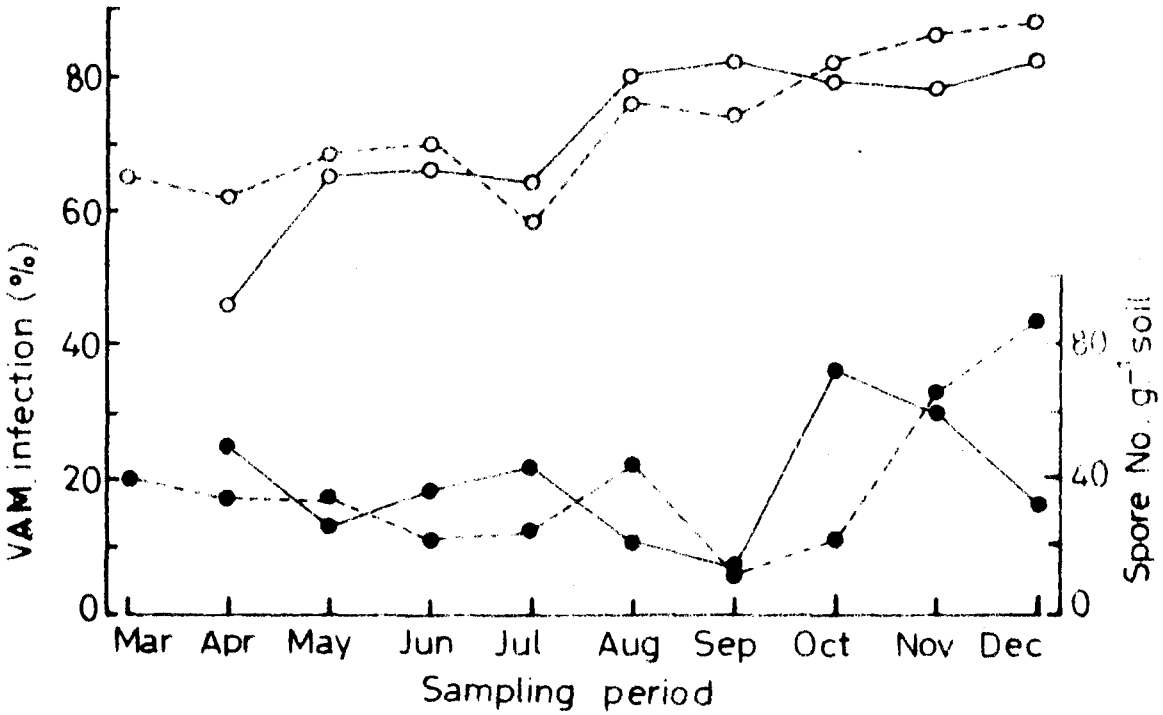


Fig. 1.3 Relationship of the average temperature with the percentage occurrence of the "fine endophyte" in the root of *E. adenophorum* (a) and *E. riparium* (b) in one year cycle (1980-81). o—o, average temperature.

Fig- 1.3

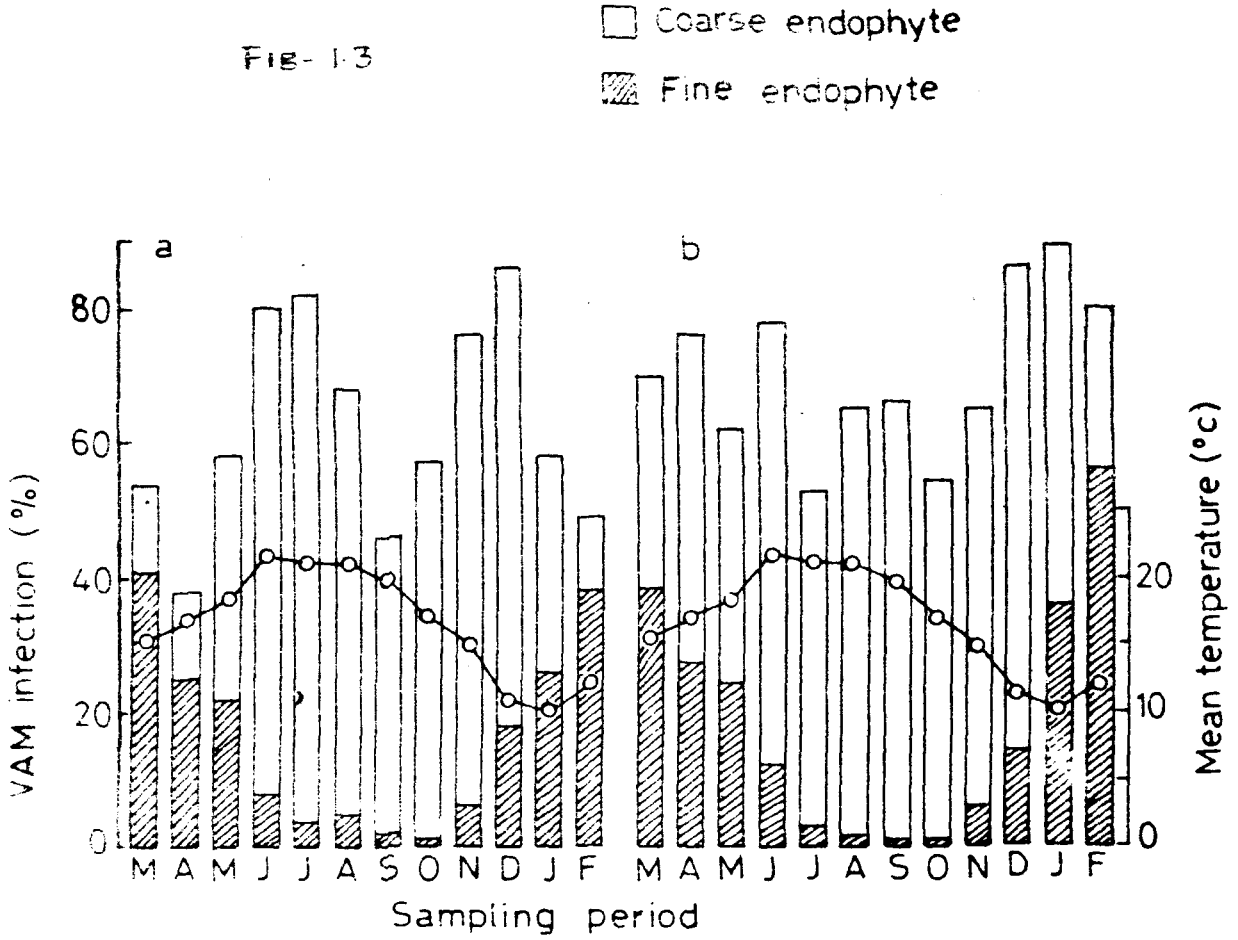

Coarse endophyte
Fine endophyte
a
b
VAM infection (%)
Mean temperature (°c)
0
20
40
60
80
0
10
20
M A M J J A S O N D J F M A M J J A S O N D J F
Sampling period

Fig. 1.4 The total fungal population in the rhizosphere of *E. adenophorum*, *E. riparium* and *O. crinita* during 1979-80.

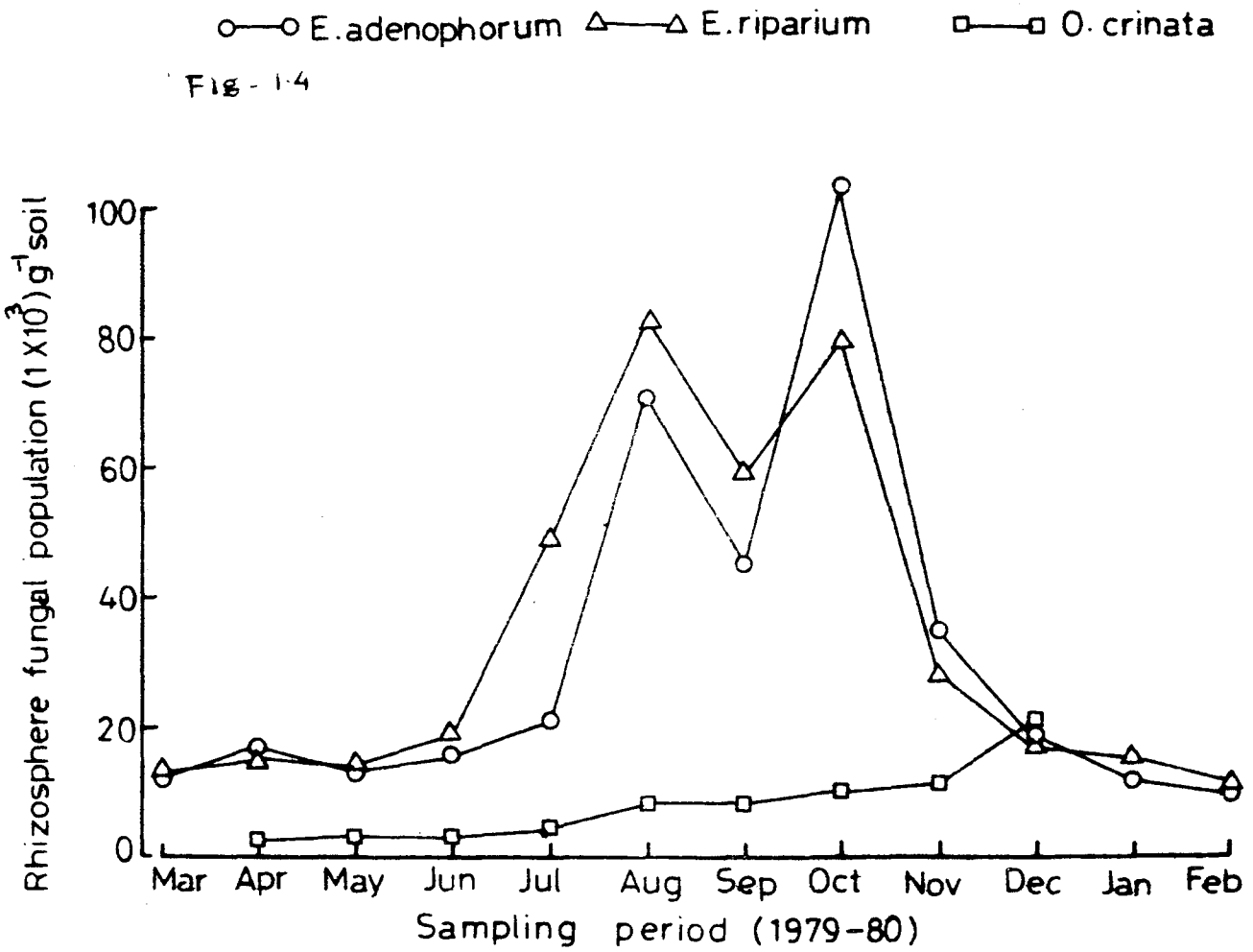

E.adenophorum
E.riparium
O. crinata
Fig - 1.4
Rhizosphere fungal population (1 X 10^3) g^{-1} soil
100
80
60
40
20
0
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Sampling period (1979-80)

Table:1.1. Rhizosphere soil properties of *Eupatorium adenophorum*

Sampling months	pH		Moisture content (%)		Organic matter (%)		Total Nitrogen (%)		Available Phosphorus (ppm)		Exchangeable Potassium (mg/g)	
	I	II	I	II	I	II	I	II	I	II	I	II
June	5.80	5.95	33.33	58.73	2.75	3.41	0.25	0.19	10.60	5.98	0.33	0.19
July	5.46	5.46	56.47	35.14	4.47	2.98	0.27	0.21	2.52	4.40	0.21	0.15
August	5.60	5.91	68.63	21.21	4.98	2.22	0.31	0.10	6.60	10.70	0.30	0.14
September	5.47	5.60	72.31	27.22	4.78	1.84	0.29	0.20	5.20	4.73	0.41	0.12
October	5.60	5.76	36.61	32.14	3.80	3.32	0.24	0.20	2.52	4.40	0.17	0.14
November	5.58	6.20	27.30	36.99	4.45	4.19	0.29	0.22	5.20	5.51	0.22	0.15
December	5.55	5.80	40.84	31.22	4.65	3.72	0.28	0.19	5.20	4.80	0.23	0.13
January	6.10	5.97	7.18	19.15	4.23	2.69	0.30	0.17	10.60	6.62	0.28	0.14
February	6.30	5.98	8.93	21.05	4.75	2.54	0.28	0.15	9.45	5.75	0.24	0.15
March	6.55	6.07	5.26	21.95	4.35	2.28	0.31	0.11	5.99	5.51	0.29	0.17
April	5.80	5.86	33.33	17.65	2.20	2.83	0.21	0.12	3.62	3.94	0.19	0.28
May	5.70	6.10	21.21	54.80	3.10	2.93	0.20	0.14	3.31	12.78	0.25	0.23

Table: 1.2 Rhizosphere soil properties of Eupatorium riparium

Sampling months	pH		Moisture content (%)		Organic matter (%)		Total Nitrogen (%)		Available Phosphorus (ppm)		Exchangeable Potassium (mg/g)	
	I	II	I	II	I	II	I	II	I	II	I	II
June	5.73	5.50	39.08	42.86	2.26	3.52	0.22	0.19	6.93	7.10	0.31	0.17
July	5.80	5.50	49.13	26.58	4.26	3.78	0.21	0.19	2.76	2.68	0.18	0.18
August	5.40	5.80	57.23	21.35	4.60	3.15	0.21	0.20	4.72	6.62	0.13	0.16
September	5.63	5.60	40.84	27.22	3.0	1.84	0.23	0.20	5.20	4.73	0.17	0.12
October	6.0	5.50	52.43	28.15	3.90	3.20	0.25	0.20	7.70	4.40	0.20	0.14
November	6.10	5.70	29.87	25.0	3.66	3.10	0.19	0.14	3.62	5.51	0.17	0.13
December	6.10	5.76	32.10	23.0	3.22	3.88	0.25	0.16	2.09	8.03	0.23	0.15
January	6.30	5.87	23.15	19.15	4.45	2.69	0.32	0.17	8.66	6.62	0.23	0.14
February	5.85	5.98	11.48	20.05	4.15	2.60	0.28	0.14	5.50	5.80	0.20	0.15
March	6.10	5.90	11.11	28.21	4.17	4.14	0.21	0.12	7.70	2.21	0.17	0.18
April	5.79	6.03	25.0	21.95	2.87	3.55	0.23	0.17	3.0	3.62	0.24	0.25
May	5.60	5.90	21.21	54.80	2.0	3.10	0.19	0.20	3.0	9.61	0.30	0.25

I = (1979-80); II = (1980-81).

Table: 1.3 Rhizosphere soil properties of Osbeckia crinita

Sampling months	pH		Moisture content (%)		Organic matter (%)		Total Nitrogen (%)		Available Phosphorus (ppm		Exchangeable Potassium (mg/g)	
	I	II	I	II	I	II	I	II	I	II	I	II
March	5.30	-	16.27	-	4.75	-	0.30	-	3.86	-	0.14	-
April	4.90	5.56	14.94	23.46	2.30	4.09	0.21	0.23	7.70	3.94	0.15	0.20
May	5.30	5.70	15.87	31.41	2.68	3.40	0.19	0.13	13.90	6.62	0.18	0.10
June	5.33	5.50	29.53	33.33	4.10	3.76	0.24	0.18	4.33	5.51	0.44	0.10
July	5.30	5.50	43.21	26.58	4.24	3.78	0.25	0.19	4.33	2.68	0.18	0.18
August	5.20	5.51	41.44	18.76	4.91	3.88	0.31	0.16	4.72	4.40	0.17	0.10
September	5.30	5.60	44.92	27.22	4.48	3.26	0.28	0.14	6.93	5.98	0.19	0.07
October	4.92	5.50	17.23	23.10	3.05	3.26	0.30	0.19	13.78	4.42	0.15	0.05
November	5.20	5.30	12.35	11.11	4.90	4.60	0.38	0.23	9.92	5.97	0.18	0.12
December	5.33	5.40	24.68	12.10	3.42	3.40	0.28	0.18	3.94	8.03	0.17	0.13

I = (1979-80); II = (1980-81).

Table: 1.4 Relationship (r) of the spore population, the mycorrhizal infection with rhizosphere soil properties and the climatic factors in *Eupatorium adenophorum*

	Rhizosphere soil properties						Climatic factors		
	pH	MC	OM	N	P	K	Max. T	Min. T	Rain-fall
Spore population									
1979-80	-0.432	0.246	0.411	0.359	-0.012	-0.146	-0.348	-0.251	-0.251
1980-81	0.099	0.109	0.741**	0.625*	-0.490	-0.119	-0.391	-0.301	-0.291
Infection percentage									
1979-80	0.167	-0.486	-0.111	-0.064	0.146	-0.423	-0.597*	-0.648*	-0.470
1980-81	-0.284	-0.184	0.066	-0.150	-0.108	-0.065	-0.655*	-0.593*	-0.403

MC = Moisture content, OM = Organic matter, N = Nitrogen, P = Phosphorus, K = Potassium, T = Temperature.

* Significant at 5% level; ** Significant at 1% level.

Table: 1.5 Relationship (r) of the spore population, the mycorrhizal infection with rhizosphere soil properties and the climatic factors in Eupatorium riparium

	Rhizosphere soil properties						Climatic factors		
	pH	MC	OM	N	P	K	Max. T	Min T	Rain-fall
Spore population									
1979-80	0.576*	0.040	0.584*	0.373	0.144	-0.532	-0.651*	-0.547	-0.517
1980-81	0.196	-0.151	0.239	-0.094	-0.361	0.342	0.118	-0.079	-0.060
Infection percentage									
1979-80	0.527	-0.168	-0.013	0.589*	-0.087	0.393	-0.654*	-0.697**	-0.338
1980-81	-0.552	0.124	0.438	0.065	0.280	-0.260	-0.042	0.175	0.023

MC = Moisture content, OM = Organic matter, N = Nitrogen, P = Phosphorus, K = Potassium, T = Temperature

* Significant at 5% level; ** Significant at 1% level.

Table: 1.6 Relationship (r) of the spore population, the mycorrhizal infection with rhizosphere soil properties and the climatic factors, in *Osbeckia crinita*

	Rhizosphere soil properties						Climatic factors		
	pH	MC	OM	N	P	K	Max. T	Min. T	Rain-fall
Spore population									
1979-80	0.183	-0.387	0.084	0.391	-0.205	-0.263	-0.790**	-0.819**	-0.735**
1980-81	-0.50	-0.284	0.341	0.756*	-0.306	0.077	-0.154	-0.219	-0.375
Infection percentage									
1979-80	-0.035	-0.195	0.148	-0.640*	0.215	0.069	-0.620*	-0.532	-0.510
1980-81	-0.367	-0.439	-0.299	-0.364	0.451	0.719*	-0.321	-0.091	-0.335

MC = Moisture content, OM = Organic matter, N = Nitrogen, P = Phosphorus, K = Potassium, T = Temperature.

* Significant at 5% level; ** Significant at 1% level.

Table ; 1.7. List of fungi present in the rhizosphere.

Fungi isolated	JAN			FEB			MAR			APR			MAY			JUN			JUL			AUG			SEP			OCT			NOV			DEC		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Absidia spinosa Lendner.	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Acremonium sp.	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Alternaria alternata	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
A. tenuis Nees.	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascomycetes	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus niger Van Tiegh.	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	+	-	+	-	-	+	+	+	+	+	+	+
Aspergillus sp.	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aureobasidium sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Cephalosporium sp.	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	-	+	+	-	+	-	+	-	-	-	+	-	+	-	-	+	-	-	+	-	-
Chaetomium sp	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium sp.	-	+	+	-	+	+	+	-	+	+	+	+	-	+	+	-	+	+	-	-	+	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+
Geotrichum sp.	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
Isaria sp.	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Mucor sp.	-	-	-	-	+	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-
Monilia sp.	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Paecilomyces sp.	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Papularia sp.	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phoma humicola Grimes. Connor and Cummins.	-	+	+	-	+	+	-	+	+	-	-	-	-	+	-	-	+	+	-	-	+	+	-	+	-	-	+	-	-	-	+	+	+	+	+	+

(Contd----)

Table : 1.7 (contd.)

Fungi isolated	JAN			FEB			MAR			APR			MAY			JUN			JUL			AUG			SEP			OCT			NOV			DEC		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Penicillium fumigatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Penicillium sp sp. 1	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Penicillium sp. 2	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pythium sp.	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-
Rhizopus sp.	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Scopulariopsis sp.	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Trichoderma viride Pers.Ex. Gray.	-	+	+	-	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Verticillium sp.	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
Yeasts 1	-	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Yeast filamentous 2	-	+	-	-	+	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
Sterile white mycelia	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Sterile Black mycelis	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	+	-	+	-	+	-	-	-	-	+	-	-	-	+	+	-	-	+	+	+	+

A = Osbeckia crinita; B = Eupatorium adenophorum; C = Eupatorium riparium.

Discussion

The highest infection level was achieved by all the three plant species in winter months as has also been reported by Sparling and Tinker (1978a). The life cycle of the Osbeckia crinita is completed within 8 or 9 months between March/April and December. The mycorrhizal infection in this plant species increased with the age of the plants (Hayman, 1970). The age of the plant was not related to the infection level in case of E. adenophorum because the higher infection level was maintained throughout the year (Fig. 1.1.a). However, in E.riparium comparatively lower infection percentage was noticed at seedling stage around March/April. Although both the species of Eupatorium are found mostly under similar conditions in nature, therefore, the difference in the infection pattern among them should be attributed to the individual plant character.

The seasonal influence on the dominance of the "fine endophyte" (Glomus tenuis) was interesting. It appeared that the lower temperature in the winter months favoured the activity of G. tenuis because the negative correlation between the infection percentage by this endophyte and the average temperature (—0.589 and —0.610 in case of E. adenophorum and E. riparium respectively) was found significant at 5% level (Fig. 1.3 a,b). The occurrence of fine endophytic infection (Rhizosphagus tenuis) in tussock grasses in New Zealand at higher altitude has also been reported by Crush (1973). The

presence of G. tenuis as the dominant endophyte in most of the plant species of higher alpine vegetation (Haselwandter and Read, 1980), and the dominance of the G. tenuis in winter months in case of Eupatorium species, suggest that the activity of G. tenuis is mostly favoured by lower temperature.

Although not presented in data, it was observed that the external vesicles (formed by Gigaspora sp.) always appeared during summer months (between March to October). It can be assumed that the higher temperature of the environment favoured the activity of the Gigaspora sp. The co-existence of the fine endophyte as well as the coarse endophyte has also been reported by Ali (1969) and Crush (1973).

The Endogone spore population found in the rhizosphere of all the three plant species was quite within the range of what has been reported by others (Mosse, 1973a). However, no clear trend in spore population could be obtained in case of E. adenophorum and E. riparium and also the correlations with most of the edaphic characters were not found to be significant statistically. The dominance of the G. tenuis during winter months may be considered to be important factor behind this because, G. tenuis produces such a small spores which cannot be recovered on the sieves of 50 μ pore size (Hayman, 1978). The geographical conditions of the north-east region and the agriculture practice followed by the local traibals could also be considered. The shifting cultivation practice, exposes the top soil to heavy erosion due to high annual

rainfall. Therefore, the water flow carries the soil particles, the organic matter and the nutrients along with the Endogone spores because most of the spores are present only in upper 15 cm depth of soil (Mosse, 1973a). Probably this may be one of the reasons as to why most of the correlations between the spore population as well as the percentage infection were not found to be significant with respect to soil properties. On the other hand the climatic factors such as temperature (maximum and minimum) and the rainfall showed the negative relationships with the spore population and the mycorrhizal infection which were sometimes found significant at 1% level also. Therefore, it can be concluded that the rainfall and the temperature fluctuation which are controlling environmental factors of this region have marked influence on the mycorrhical status of the soil and the plant species. As regards the "rhizosphere effect", it has been reported that the spore population is not related to the host species (Kruckelman, 1975). The general increasing trend of the spore population in the root region of the Osbeckia crinita may be attributed to its annual character because in the later half of the life cycle when the roots were dying the increase in spore population was expected (Hayman, 1978).

The increase in population of rhizosphere fungi during July-November in case of E. adenophorum and E.riparium may be attributed to the high moisture and comparatively warm atmospheric conditions which would have favoured the greater

fungal activity either directly or through the "rhizosphere effect", because that period happens to be the greater growth period of both the Eupatorium species. However, in case of Osbeckia crinita, higher fungal population was recorded in the later half of the life cycle. The contribution of the dead roots in the surrounding may be the reason of the highest fungal population in the month of December (Alexander, 1978), when the plants die after flowering and fruiting.

CHAPTER II

The comparative studies of the Vesicular-arbuscular mycorrhizal status of two contrasting sites.

Introduction

The ubiquitous nature of vesicular-arbuscular type of mycorrhiza is well known (Mosse, 1973; Gerdemann, 1975). The mycorrhizal association with the root system is so prevalent that it is difficult to separate the non-mycorrhizal root from the natural conditions. Wilhelm (1966) stated truely that under field conditions, plants do not strictly speaking have roots—they have mycorrhiza.

The shifting cultivation which is common in north-east India, has disturbed the lands of this region to a great extent. The frequent burning of the above ground vegetation has its adverse effect on the normal root production capacity of the soil. The natural roots are the sites of mycorrhizal proliferation and the network of the mycelial extensions of the mycorrhizal fungi beyond the root surface in addition to other soil fungal species are reported to be responsible for the aggregation of sandy soils (Bond, 1960; Bond and Harris, 1964; Thornton et al., 1956; and Sutton and Sheppard, 1976). The hilly slopes of this region, which receives very high rainfall annually, have created a favourable condition for the accelerated soil erosion, particularly when the aggregating power of the soil has been reduced considerably.

During the process of soil erosion not only the fertile soil but the mycorrhizal spores (which are mostly found in the upper soil horizons) are also washed along with the eroded soil. Therefore, it was thought relevant to assess the mycorrhizal loss in such a disturbed situation. The present work deals with the comparative study of two sites with respect to seasonal changes in root production, the nutrients concentration, with special reference to the mycorrhizal infection and the mycorrhizal spore populations at different soil depths.

Material and Methods

Two contrasting sites, one open, upland and the other closed, down the hills, were selected. The open site designated as Site I was under frequent disturbance of burning and was characterised by the very sparse overstorey of Pinus kesiya with the understorey dominated by the herbaceous weed species like Osbeckia crinita, Arundinella benghalensis, Imperata cylindrica and Launea sp. On the other hand, the closed site designated as Site II, was an old forested fallow with the dense Pinus kesiya as the dominant tree component of the overstory and Eupatorium adenophorum and E. riparium as the dominants of understory.

The sampling area of 20 m X 20 m in both the sites were marked and the soil samples were collected from six depths (0-5, 5-10, 10-15, 15-20, 20-25 and 25-30 cms) and six randomly selected spots. The sampling was started in October, 1980

and four samplings were done in order to cover four seasons. January represented the winter, April, the spring, July, the rainy and October, the autumn seasons. All the samplings were done at the end of the respective months. It is important to mention here that Site I was subjected to burning in the third week of March 1981, and hence the sampling of April was done after five weeks of burning.

Further, from each lot of soils of different depths, 50 g soil with intact root systems was weighed in duplicate and from the weighed samples the root materials were taken out carefully by small forceps under very shallow water in a tray. These roots were thoroughly washed in water and weighed after being blotted between the filter papers. The ectomycorrhizal roots and also the dead roots (distinguished morphologically, by the presence of dichotomous branching and black colour respectively) were removed before weighing. Later on each replicate of roots of different depths were mixed together separately. However, the root seperation on individual plant species level could not be done in order to minimise the loss of fine roots (Sparling and Tinker, 1978a). $\frac{1}{2}$ Kg soil samples representing each replicates were further mixed together and except a small amount which was taken for the pH and moisture content determination, the rest was air dried before the Endogone spores were isolated and the nutrient analyses were done.

For the estimation of mycorrhizal infection in the root system, comparatively thinner roots were cleared, stained

(Phillips and Hayman, 1970) and observed under microscope. In case of lower depths, the root materials were considerably less, particularly at Site II, hence only 40-60 root segments could be observed.

The Endogone spores were isolated from 10 g air dried soil in triplicate following the method wet-sieving and decanting (Gerdemann and Nicolson, 1963) and the spores retained on the sieve pore size of 150 μ, 90 μ and 50 μ were separately filtered on the filter paper (Whatman No.1). The spores were counted under simple stereomicroscope.

The following methods were followed for the estimation of nutrients concentration of soil: Walkely Black method for organic matter, Micro-Kjeldahl method for total nitrogen and Molybdenum blue method for available phosphorus - as outlined by Jackson (1973). The potassium was extracted in ammonium acetate solution (pH - 7) and read in flame photometer, following Allen (1974).

Results

Seasonal variation:- The root weight in the April sample of Site I which was taken after 5 weeks of burning was significantly less than others. Afterward the root weight showed a continuous increase along the seasons and was maximum in January (Fig. 2.1b). The highest peak in root production was observed in October at Site II but an abrupt fall was recorded

in July (Fig. 2.1b). The vesicular-arbuscular mycorrhizal infection followed almost the pattern of root weight in all the seasons and at Site II it showed a significant ($P > 0.05$) positive correlation ($r = 0.94$) to each other (Fig. 2.1b).

The spore population exhibited the reverse trend and generally Site I harboured less number of spores than Site II. In the rainy season (July) the spore population was higher at Site II but less at Site I. The seasonal fluctuation of spore population showed reverse trend at both the sites (Fig. 2.1c). The analysis of spore size revealed that the majority of the spores, were of the size $> 90\,\mu$, followed by $> 50\,\mu$ and least were the spores $> 150\,\mu$ (Fig. 2.1a), in the total spore populations.

The soil of both the sites was acidic (Fig. 2.2e). The moisture content of the soil at both the sites was lowest in January and highest in July but comparatively higher moisture percentage was recorded in Site II than Site I in all the seasons (Fig. 2.2.f). The soil organic matter, the total nitrogen and the available phosphorus of one site was found negatively correlated to other. Two maxima, one in April and another in October were observed for organic matter and nitrogen at Site I while in contrast to this, two depressions were noted at Site II during the same period (Fig. 2.2c,d). The maximum peak in available phosphorus was achieved in July at Site I and in April at Site II but the corresponding concentrations of the same at both the sites were generally reverse to each

other (Fig. 2.2.a). The maximum potassium peak was observed in July and also their fluctuation in concentrations due to season were similar at both the sites (Fig. 2.2.b). In general, the concentration of all the nutrients was higher at Site II and the seasonal effect was more pronounced at Site I.

Depth-wise variations:- The decrease in root weight along the depth was common at both the sites (Fig. 2.3). However, the total root production was significantly greater ($P > 0.05$) at Site I than Site II throughout the seasons along the depths, except at lower depths (20-30 cms). At Site I, the decreasing order of root weight was significant ($P > 0.05$) in upper 3 depths (0-15 cms) and in two depths (0-10 cms) at Site II. The mycorrhizal infection percentage also showed the similar decreasing trend along the depths but it was always significantly higher ($P > 0.05$) at Site I in comparison to Site II (Fig.2.3). The range of percentage infection was 85-47 in Site I and 57-13 in Site II. No infection was recorded in the root at lower two depths (20-30 cm) in Site II in rainy season (July).

The total Endogone spores, also followed the decreasing trend along the depths except at 0-5 cm. depth at Site II when it exhibited the unusual lower numbers than 5-10 cm. depth (Fig. 2.4). The spore population was significantly higher ($P > 0.05$) at Site II, in general, except at upper layer (0-5 cm). Here again, it was clear that the spores ranging in size between 150 μ and 90 μ constituted the major portion of the total spore numbers followed by the size

between 90 μ and 50 μ. The spores $> 150\ \mu$ were least in number. However, the decreasing order of the spores were significant ($P > 0.05$) upto 20 cm depth in October, upto 15 cm in January and July but only upto 10 cm in April in case of Site I whereas at Site II on the other hand it was significant upto 20 cm depths in October, upto 15 cm in April and upto 10 cm in January and July.

The phosphorus and potassium concentrations in soil showed a decreasing trend with the increasing depths at both the sites (Fig. 2.5 a,b). The overall concentration of phosphorus however, was always higher at Site I than Site II while the concentration of potassium was higher at Site II in comparison to Site I at all the depths.

The trend of organic matter and nitrogen concentration was quite different in Site II while in Site I it followed the usual decreasing trend (Fig. 2.5 c,d). The organic matter was only slightly less at 0-5 cm depth in Site II which increased further and remained nigh and almost unchanged at all the depths. The nitrogen concentration in Site II decreased at 5-0 cm depth but afterwards it showed an increasing trend along the depths.

Discussion

Nutrient concentration was generally highest at upper horizon (0-5 cm). At Site II the high nitrogen and organic

Fig. 2.1 a. Seasonal variation in the *Endogone* spore population of different size categories present vertically in the soil upto 30 cm depth at Site I and Site II.

b. Seasonal variation in the root weight and the percentage VA-mycorrhizal infection of the root present vertically in the soil upto 30 cm depth at Site I and Site II. △——△ , mycorrhizal infection at Site I; ▲——▲ , mycorrhizal infection at Site II; O——O, root weight at Site I; ●——●, root weight at Site II.

c. Seasonal variation in the *Endogone* spore population present vertically in the soil upto 30 cm depth at Site I and Site II. O——O, spores at Site I, ●——●, spores at Site II.

Fig. 2.1

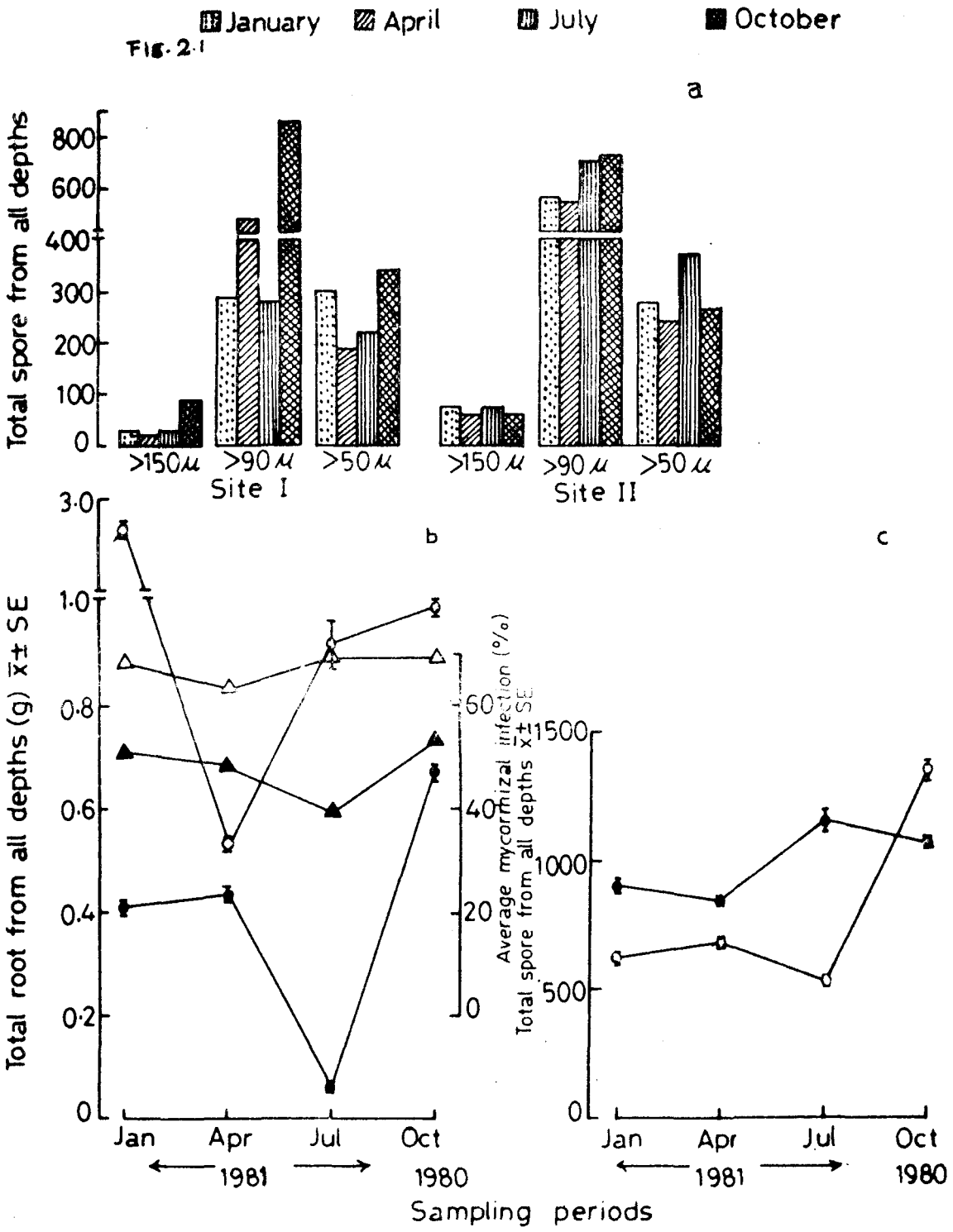

January
April
July
October
a
Total spore from all depths
800
600
400
300
200
100
0
>150μ
>90μ
>50μ
Site I
Site II
b
c
Total root from all depths (g) x̄ ± SE
3·0
1·0
0·8
0·6
0·4
0·2
0
60
40
20
0
Average mycorrhizal infection (%)
Total spore from all depths x̄ ± SE
1500
1000
500
0
Jan
Apr
Jul
Oct
1981
1980
Sampling periods

Fig. 2.2 Seasonal variation of the nutrients concentration in the soil of Site I and Site II. O——O, Site I; ●——●, Site II.

Fig. 2.2

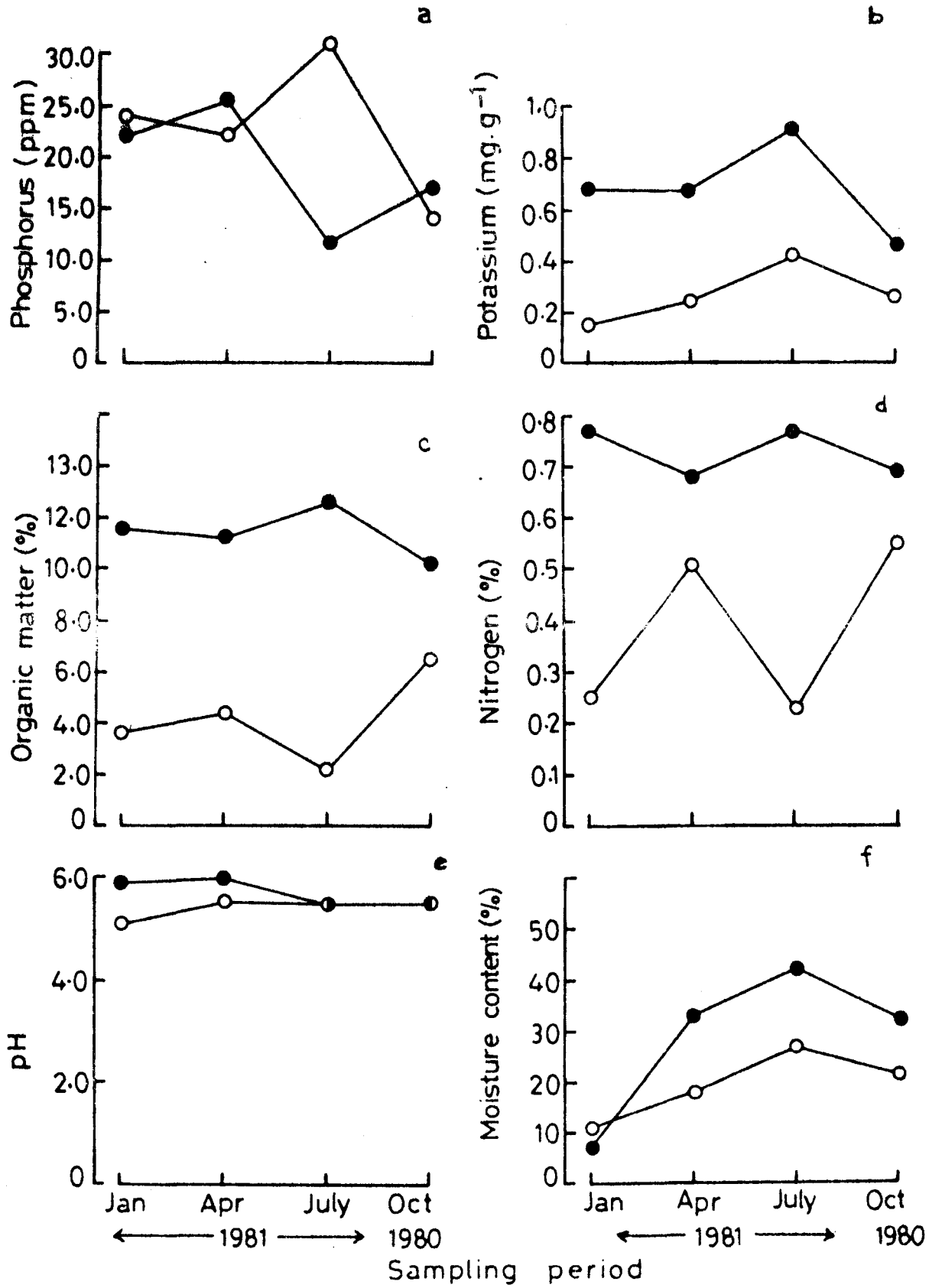

a
b
c
d
e
f
Phosphorus (ppm)
30.0
25.0
20.0
15.0
10.0
5.0
0
Potassium (mg.g⁻¹)
1.0
0.8
0.6
0.4
0.2
0
Organic matter (%)
13.0
12.0
10.0
8.0
6.0
4.0
2.0
0
Nitrogen (%)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
pH
6.0
4.0
2.0
0
Moisture content (%)
50
40
30
20
10
0
Jan
Apr
July
Oct
1981
1980
Sampling period

Fig. 2.3 Depth-wise variation in the amount of root and the percentage VA-mycorrhizal infection of the root, present in the soil of Site I and Site II.

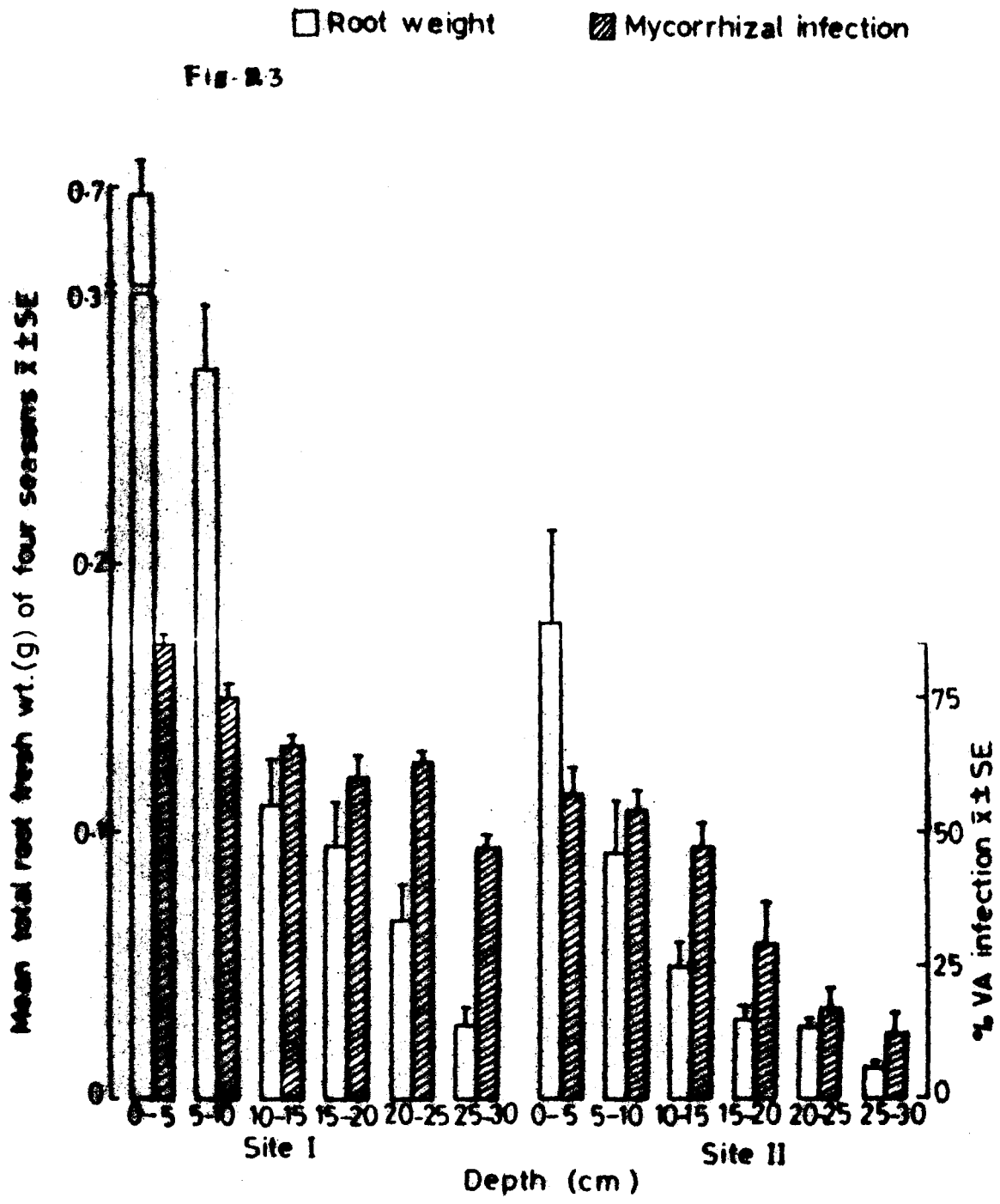

Root weight
Mycorrhizal infection
Mean total root fresh wt.(g) of four seasons x̄ ± SE
0·7
0·3
0·2
0·1
0
% VA infection x̄ ± SE
75
50
25
0
0–5
5–10
10–15
15–20
20–25
25–30
Site I
Site II
Depth (cm)

Fig-2.3

Fig. 2.4 Depth-wise variation of the Endogone spores of different size categories in the soil of Site I and Site II.

Fig-2.4

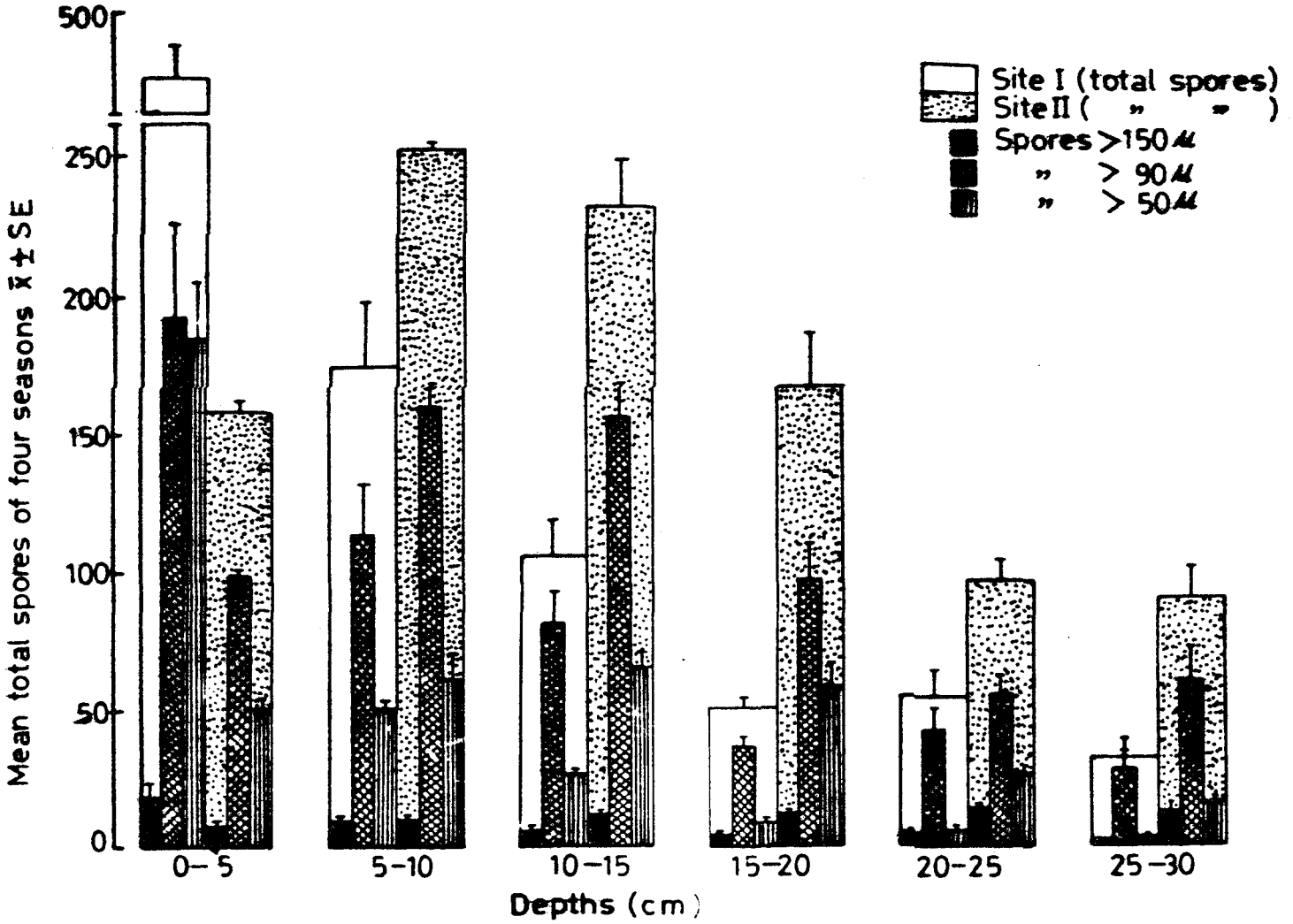

Site I (total spores)
Site II (" ")
Spores >150 μ
" > 90 μ
" > 50 μ
Mean total spores of four seasons X̄ ± SE
500
250
200
150
100
50
0
0–5
5–10
10–15
15–20
20–25
25–30
Depths (cm)

Fig. 2.5 Depth-wise variation of the nutrients concentration in the soil of Site I and Site II.

Fig-2.5

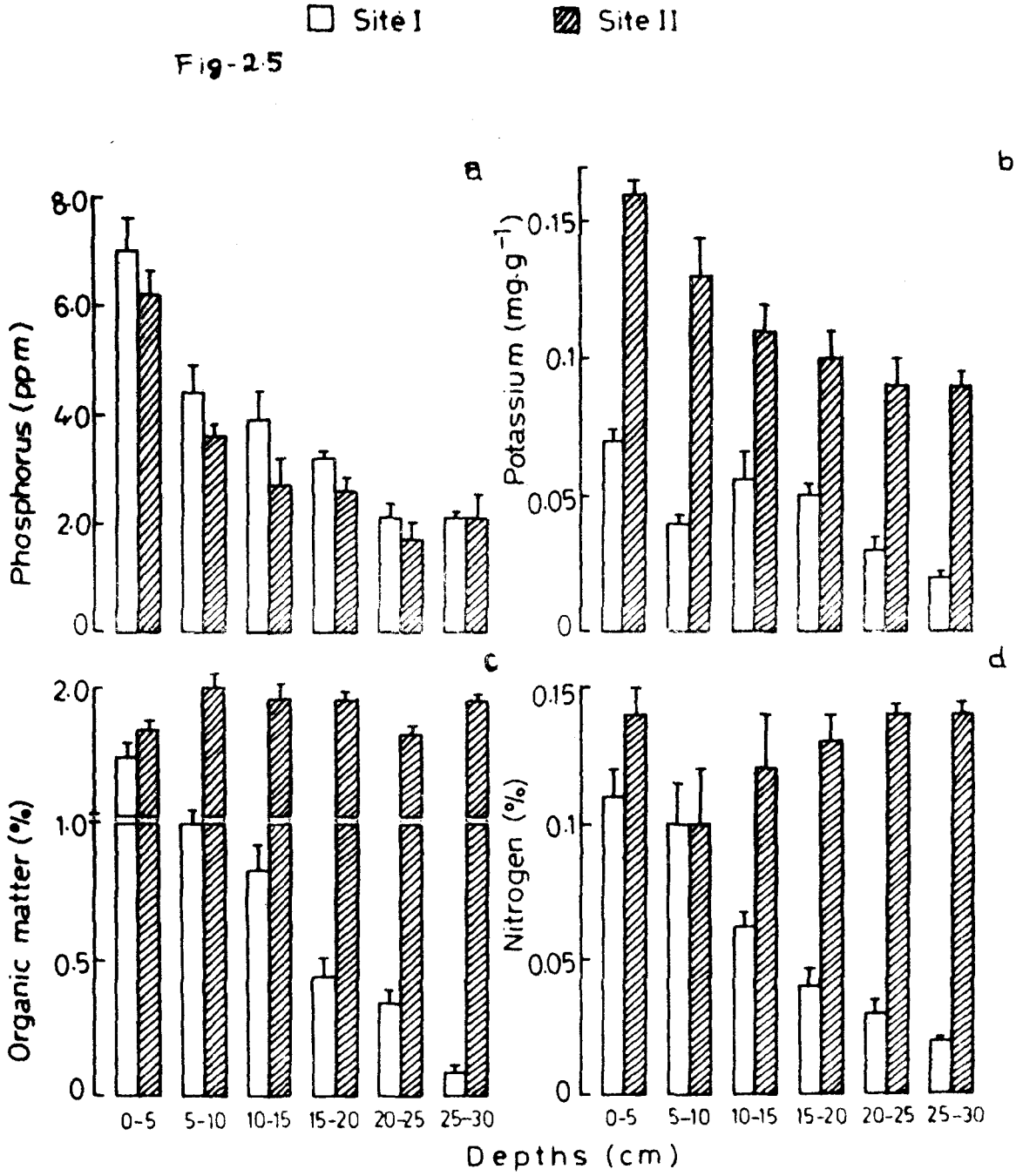

Site I
Site II
a
b
c
d
Phosphorus (ppm)
Potassium (mg·g⁻¹)
Organic matter (%)
Nitrogen (%)
8.0
6.0
4.0
2.0
0
0.15
0.1
0.05
1.0
0.5
0-5
5-10
10-15
15-20
20-25
25-30
Depths (cm)

Table:2.1 The correlation-coefficient value (r) of the Endogone spore population and nutrient concentrations of 4 seasons: (d.f = 3)

		Organic matter	Nitrogen	Phosphorus	Potassium
Spore population	Site I	0.936*	0.750^{NS}	-0.914*	-0.436^{NS}
	Site II	0.2632^{NS}	$0;346^{NS}$	-0.9916**	0.280^{NS}

* Significant at 5% level, ** Significant at 1% level.

NS = Not significant.

matter content at all the depths was probably due to the continuous deposition of the eroded soil rich in organic matter brought by water current from up the hills, Similar reason may be attributed to the generally high nutrient concentration at Site II than Site I. However, the available phosphorus was always higher at Site I at all the depths which was probably due to the greater microbial activity and release of soluble phosphate because of the open light and high temperature condition of the site. The importance of top soil in maintaining the fertility level is well known. The surface layer of soil is continuously enriched by the organic matters deposited through surroundings and the nutrient status of the belowground soil also greatly depends on the mineralization process at the top because the same released nutrients reach down the profile along with the water movement (Gupta and Rorison, 1975).

The depthwise decrease in the root weight at both the sites was similar to the observations of Sparling and Tinker (1975, 1978a). The decreasing trend of mycorrhizal infection along the depths, however, was contrary to Sparling and Tinker (1975), who found little difference in infection level upto 25 cm. depth but beyond that they also noticed a decline in infection. In general, the nutrients concentrations, mycorrhizal infection, as well as Endogone spore population decreased with depth, except in the case of organic matter and nitrogen content of the soil of Site II. The Endogone spores were higher in upper depths. Mosse (1973a) also found the maximum spore numbers at

upper 15 cm depths. The decrease in spores number along the depth was also recorded by Sutton and Barron (1972) and Schwab and Reeves (1981). The unusual less number of spores at upper horizon of soil (0-5 cm) at Site II may be attributed to the death of spores either by predation or parasitism or spore dispersal into or out of the soil volume (Janos, 1975).

The reduction in root weight during the peak season of growth i.e. in April (spring) in case of Site I was probably due to the disturbance caused by burning in the third week of March. In general, the rainy season (July) was found to have greater effect on the root production, the mycorrhizal infection, the spore population and in regulating the nutrient concentrations. The exceptionally low root weight followed by reduction in mycorrhizal infection found in July was also probably due to high moisture of the soil. Although, not significant, but a negative correlation was found between the moisture content and the root weight in different seasons. The nutritional status, except phosphate of Site II was very high in the month of July, but still the root production and mycorrhizal infection was very less. The remarks of Keely (1980), with reference to Mosse (1973a) "the increased soil nutrients will tend to reduce mycorrhizal formation since the plant is less dependent on the fungus for obtaining adequate nutrient supplies", seems to hold good for this. Another reason may be the limitation of oxygen due to water filled pore space of the soil at Site II and in that condition the mycorrhizal fungi which are aerobic by nature will have to depend on host for

oxygen demand (Read and Armstrong, 1972). The moisture induced reduction in mycorrhizal infection was also reported by Redhead (1975). Therefore, under such a stressed condition the reduction in root growth followed by the reduction in mycorrhizal infection may not be unusual as both the partners of the symbiosis are interdependent.

The high root weight with higher mycorrhizal infection at Site I, may be attributed to the greater mycorrhizal effect in nutrient deficient soil condition of the site (Mosse, 1973a). The constancy in mycorrhizal infection in all the seasons at Site I was probably due to some self-regulatory mechanism as suggested by Sparling and Tinker (1978a).

Except in October, the high level of infection at Site I was followed by low number of spores, which was a finding similar to Mosse and Bowen (1968) and Redhead (1971). The decrease in the available phosphorus in different seasons was coupled with an increase in the total endogone spores and this negative correlation was very significant in both the sites. However, it appeared that the higher spore population at Site II, specially in the rainy season (July) was also partly due to the deposition of the spores carried by the water flow from the upland to down the hills, because after the end of rainy season the spore numbers shoot up very high at Site I in the month of October. According to Sutton and Barron (1972) "the seasonal decline in spore populations may be accounted for by spontaneous germination or death, ingestion

by soil fauna, destruction by fungal or other parasites,or by stimulation of germination in the presence of living host".

Two peaks of nitrogen, one in spring (April) and another in autumn (October) was observed at Site I which was similar to the observation of Williams (1969). However, as the spring is immediately followed by rainy season, the increased concentration of nitrogen and organic matter of the uplands soils (Site I) were probably washed to the valley land (Site II), where the increased concentrations of these nutrients were observed in July (Fig. 2.2 c,d). Potassium concentration was also found to be affected by the rainy season. On the other hand, the fluctuation in the concentrations of phosphorus in different seasons was not like other nutrients. The concentration of the available phosphorus declined to a greater extent in the rainy season in case of Site II. The phosphate is relatively immobile (Bieleski, 1973) and thus its transport along the water flow from upland would also be relatively less. Further, the heavy shading and comparatively low temperature with excess of moisture in soils of Site II was probably the least favourable condition for the microbial release of the nutrients including phosphate. Therefore, the low concentration of available phosphorus in Site II even in rainy season was probably due to the reasons mentioned above.

The seasonal changes in the nutrients concentration were not found to be significantly correlated with mycorrhizal infection at both the sites, but rhe Endogone spore population

was positively correlated with organic matter and nitrogen in Site I (Table 2.1) which was probably due to the nutrient deficient condition of the soil. The same correlations were however, not significant in case of the nutrient rich soils of Site II. The Endogone spore population, on the other hand showed significant negative correlation with the available phosphorus at both the sites (Table 1).

CHAPTER III

The comparative study of the mycorrhizal infective potential of the soils of contrasting sites.

Introduction

The top soil contains the maximum number of spores (Mosse, 1973a) and therefore the erosion of top soils not only removes the nutrients from the soil but also removes the mycorrhizal spores, which are not less than biological fertilizer in promoting the growth of the plants in nature. The soil of the lands situated down the hills receives the mycorrhizal spores carried by water flow from uplands along with the top soils. The mycorrhizal spores present in the vertical column of the soils may also include the spores which settle down with water movement down the profile. Therefore, the infective potential of the mycorrhizal propagules, (including spores or resting hyphae) present at different horizons of soils in disturbed upland and comparatively less disturbed valley land, was assessed.

Material and Methods

The characteristics of both the sites have already been described in the chapter 2. The soils of all the six depths collected from both the sites in the month of April 1981 were filled in plastic pots (1.5 Kg capacity) after removing the roots from the soil. The quantity of soil was 1 Kg in all the pots.

The pre-germinated onion seeds were transferred to each pot at the rate of 5 per pot which were thinned to 3 per pot after one week of growth. Three replicates of pots with altogether 9 replicates of plants were maintained under glass house conditions till five weeks of growth when the harvest was taken. Plants were watered with ordinary tap water, thrice a week, during the experiment period April-June, 1981.

After the harvest, the roots were separated from the shoots and the entire roots of replicate plants were assessed for mycorrhizal infection, separately for each depths. The roots were cut into 1 cm. pieces, cleared in KOH and stained in cotton blue according to the method of Phillips and Hayman (1971). While observing the root segments for the presence or absence of mycorrhizal infection, the number of mycelium entry points, the percentage occurrence of arbuscles and vesicles, the mycelial coils formed within the root cortex and the types of endophytes, distinguished morphologically were taken into account. The "fine endophyte" was probably the strain of Glomus tenuis and the "coarse endophyte", some species of Glomus, as also observed by others (Crush, 1973, Ali, 1969).

Results

The infective potential of soils of Site I was considerably low in comparison to Site II. No infection was recorded in the soils of Site I taken from the depths between 20-30 cm. (Table 3.2). Even the plants grown in the soils

between 10-20 cm depths at Site I did not show the sign of healthy growth. On the other hand, the infective potential of the soils of Site II showed its superiority over Site I in all the respects, and although low, the infection was recorded in the soils of lower depths also. The percentage infection, the intensity of infection, the number of entry points per cm. of root, showed a decreasing trend along the depths at both the sites (Table 3.2). The "coarse endophytes" dominated over "fine endophytes" in colonizing the root cortex and the "fine endophytes" were found to be confined at upper layer of soils only in both the sites. The arbuscles and vesicles percentage, the occurrence of "coil" like structure in the root cortex showed a decreasing trend along the depths of soil at Site I but did not show any trend at Site II. The shoot weight of plants grown in the soils of Site II (Table 3.3) showed no relationship between shoot weight and mycorrhizal infection and it appeared that the hutrient rich soils of Site II did not require the mycorrhizal association to support the plant growth.

Discussion

The comparatively higher mycorrhizal infection at Site I under field conditions (Table 3.1) was most probably due to the spread of infection through infected roots and not through Endogone spores (Baylis, 1969; Sparling and Tinker, 1978a).because, in the pot experiment with the soils of Site I, a measurably poor infection was recorded (Table 3.2). The

Table: 3.1 The general mycorrhizal status and the nutrients concentrations of the soils of Site I and Site II under field conditions.

Depths (cm)	Mycorrhizal status				Soil properties							
	Infection (%)		Spore numbers ($100g^{-1}$ soil)		Organic matter (%)		Total N (%)		Available P (ppm)		Exchangeable K (mg/g)	
	I	II	I	II	I	II	I	II	I	II	I	II
0-5	84	70	2570	1750	1.86	1.44	0.16	0.14	6.62	7.25	0.07	0.15
5-10	76	71	1290	2580	1.32	2.04	0.19	0.15	3.31	4.41	0.04	0.12
10-15	68	55	920	1330	0.63	1.77	0.06	0.14	2.48	3.31	0.03	0.12
15-20	38	11	550	680	0.33	1.92	0.04	0.14	2.21	2.36	0.03	0.08
20-25	48	6	638	560	0.12	1.98	0.03	0.14	3.31	3.22	0.02	0.10
25-30	37	13	690	670	0.09	2.04	0.04	0.14	3.62	4.90	0.02	0.10

Table: 3.2 Mycorrhizal infective potential of the soils of two sites (I and II)

Parameters		Depths (cm) 0-5	5-10	10-15	15-20	20-25	25-30
% infection	I	51.0	19.30	11.70	3.30	0	0
	II	63.1	30.0	27.5	13.80	5.70	4.0
Entry points/ cm. root segments	I	3.10	2.30	1.20	0.70	0	0
	II	7.60	3.0	2.90	1.50	1.0	1.30
% arbuscles	I	64.0	56.0	33.30	9.0	0	0
	II	63.40	16.6	22.70	11.10	27.0	0
% coils (Pelotones)	I	28.60	25.50	18.0	13.40	0	0
	II	26.80	60.0	50.0	55.5	50.0	100
% vesicles	II	25.0	13.0	7.50	2.30	0	0
	II	75.60	53.30	27.30	11.10	75.0	66.60
No.of vesicles/ infected segments	I	4.96	3.60	1.30	0.40	0	0
	II	13.10	5.30	4.40	0.60	5.0	3.70
% fine endophyte	I	21.40	0	0	0	0	0
	II	4.90	10.0	14.20	0	0	0
% course endophyte	I	46.40	100	100	100	0	0
	II	68.30	76.60	58.50	100	100	100
% fine + coarse endophyte	I	32.14	0	0	0	0	0
	II	26.80	13.30	27.30	0	0	0
Intensity of infection	I	++	+	+	+	-	-
	II	+++	++	+	+	+	+

Table: 3.3 Shoot, dry weight (mg) of the onion plants grown in the soils of different depths. $\bar{X} \pm SE$

Sites	Depths (cms)					
	0-5	5-10	10-15	15-20	20-25	25-30
Site I	3.87±0.51	2.9±0.38	2.4±0.24	2.0±0.28	1.85±0.24	1.76±0.32
Site II	6.5±0.65	5.6±0.53	7.03±0.56	7.79±0.62	7.2±0.54	8.59±0.52

conditions of Site I was comparable to the "eroded soils" which are poor in mycorrhizal infection (Hall and Armstrong, 1979). The cause of disturbances in case of Site I has been explained in the chapter 2. The extremely poor performance of the mycorrhizal propagules present in the soils of Site I can be attributed to the various factors induced by disturbances, including: (i) The extremely poor nutrient status of the soils of Site I (Table 3.1) which inhibits the mycorrhizal formation as reported by Hayman (1970, 1975), Porte and Bente (1972), Mosse (1973a), Kruckelman (1975), McIlveen, Spotts and Davis (1975), who suggested that the excessive high or the extremely poor nutrient status of the soils, both are inhibitory to mycorrhizal formation. The organic material of soil also provides a base to mycorrhizal spores to propagate as saprophytes and further penetrate the new roots (Warner and Mosse, 1980).(ii) The soil moisture, the temperature, the light intensity (Hayman et al., 1976; Hayman, 1974), the various chemical factors of soil (Schwab and Reeves, 1981) and the associated microorganisms (Mosse, 1973a), all the factors are disturbed in a disturbed land, which are very important for the successful mycorrhizal establishment.

Contrary to Site I the infective potential of Site II was considerably good (Table 3.2). The VAM fungi can survive in soil as spores or hyphae (Gerdemann, 1975) and their viability is also retained in soil at least for a year (Hayman, 1975). The conditions at Site II was more congenial for the

preservation of the viable spores which was also seen in the pot experiment where the soil of the lowest depth (25-30 cm) was also able to infect the root (Table 3.2).

The dominance of the "coarse endophyte" at all the different depths soils and the confinement of the "fine endophyte" at upper depths only, revealed that the active zone of the fine endophytes are mostly the upper horizons of the soil. The number of entry points showed a decreasing trend along the increasing depths which was similar to the decreasing infection percentage probably due to decreasing order of viable spores or hyphae along the depths. The number of entry points at the upper depths of soil at Site II (Table 3.2) was similar to Sanders and Tinker (1973), who observed 6 entry points per cm root in onion. The observation of Hall (1977) that the infection in soils low in available phosphorus had more arbuscles and vesicles than the soil with higher available phosphorus, was comparable to the findings of this study. The vesicles number and their percentage occurrence showed no trend and a similar erratic behaviour of vesicles was found by Redhead (1975) in a different study. The formation of "coils" by the mycelium of the endophytes was probably host induced and not due to different mycorrhizal strains (Boullard and Ferchau, 1962; Hayman, 1975). The high nutrient levels of soil minimises the need of mycorrhizal infection (Mosse, 1973a) and therefore the "coil" formation (very high in Site II, Table 3.2) as a result of host resistance seems to

be more convincing. This was obvious from the shoot weight in the soils of Site II, which increased according to the rich soil nutrient levels along the depths (Table 3.3) and not according to the mycorrhizal infection levels (Table 3.2).

The improvement in the soil fertility for the revegetation of the disturbed lands by exploiting the rich soils of Site II conditions, would be far superior to the additions of other fertilizers because these sort of mixing the soils would not only increase the symbiotic fungal populations, but other microorganisms also, which are resoonsible for nitrogen fixation, soil aggregation and also improving the physical and chemical properties of soils (Schwab and Reeves, 1981).

CHAPTER IV

Effect of soluble phosphate (Na_2HPO_4) on the vesicular-arbuscular mycorrhiza of *Eupatorium adenophorum* Spreng.

Introduction

Graw *et al.*, (1979) included *Eupatorium odoratum* in the group of plants which depends entirely on vesicular-arbuscular mycorrhiza for their phosphate uptake and growth. Gerdemann (1975) compared the growth of mycorrhizal and non-mycorrhizal plants on different fertility levels in order to determine the mycorrhizal dependency of a particular species. The role of vesicular-arbuscular mycorrhiza on the growth and phosphate nutrition of the plant has been extensively discussed (Mosse, 1973a, Gerdemann, 1975). The decrease in the vesicular-arbuscular infection of plant roots in response to phosphate additions to soil has also been observed (Mosse, 1973b; Sanders, 1975, Abbott and Robson, 1977b; Menge *et al.*, 1978a and Jasper *et al.*, 1979). The present study was undertaken to evaluate the mycorrhizal dependency of *E. adenophorum* in relation to phosphate additions (0-5 g per pot) to soil. The role of mycorrhizae in the uptake of nitrogen and pptassium has also been discussed.

Materials and Methods

The garden soil of the following properties: organic matter 2.2%, total nitrogen 0.2%, available phosphorus 4.2 ppm, exchaggeable potassium 0.15 mg/g, and pH 5.6, was diluted 4

times by adding acid washed sand in 1:4, soil : sand ratio. This mixture was autoclaved two times at 15 lb pressure for 3 hours with an interval of 24 hours, in order to remove the mycorrhizal propagules from the soil. 3 Kg of this soil : sand mixture was put in the plastic pots and left for two weeks under moist condition to overcome any toxic effect of steam sterilization and to regain the microbial activity.

Nine concentrations of Na_2HPO_4 i.e. 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 3.0 and 5.0 gs were mixed thoroughly in separate pots. 8 pots were used for each concentration of phosphate. 4 of these pots for each concentrations were used as 4 replicates for mycorrhizal treatments and remaining 4 for the non-mycorrhizal treatments.

The local isolate of mycorrhizal fungi Glomus tenuis, isolated and maintained on the host Eupatorium riparium was used as mycorrhizal inoculum. 50 ml of the inoculum containing roots and soil was evenly spread below 3 cm of the soil surface in the pots meant for the experiment of mycorrhizal treatments. The remaining pots to be used for non-mycorrhizal experiments received the twice filtered washings of the same inoculum in order to keep the other microbial characters similar in both the sets.

The seeds of E. adenophorum were germinated on moist filter paper in petriplates at 30°C in an incubator and 6 numbers of 5 days old seedlings were transplanted in every pots,

which were finally thinned to 3 per pot after one week of growth. The experiment was conducted under glass house conditions during the months April-August 1981. The pots were watered at every alternate day for 4 weeks and then twice a week till the end of the experiment. The plants were harvested after 16 weeks.

Measurements:- The shoot and root length was measured by general scales. The diameter of 1st internode of stem was measured by slide callipers. The leaf area was calculated on per plant leaf dry weight basis, after determining the total area of leaves of a plant by planimeter. The dry weight was obtained after drying the plant materials at 80°C oven and reweighing till constant weight.

Percentage infection:- For the measurement of the percentage infection, the roots of approximately 1 cm. were cleared in KOH and stained with cotton blue, following the methods of Phillips and Hayman (1970). 70 to 100 root segments were observed under microscope, based on the quantity of roots, for the presence or absence of infection.

Chitin assay:- The chitin assay of the infected roots, was done according to the methods of Tsuji et al., (1969) and Ride and Drysdale (1972). The roots were washed carefully in the tap water and a portion of them was cut into small pieces and blotted between filter papers. 100 mg of the blotted roots were weighed in duplicate one for chitin assay and another for dry weight determination. For the chitin assay, the roots were

grinded in mortar and pestle with acetone, then washed with distilled water and boiled in concentrated KOH (120 g dissolved in 100 ml) at 130°C for 90 minutes. Alkali was removed further with the help of 75% and 40% ethanol. Subsequently removal of alkali was done through centrifuging technique. The residue, in the form of 'chitosan' was deaminated with $NaNO_2$, $KHSO_4$ and sulfamate ($NH_4SO_3NH_2$) which was finally reacted with 3-methyl - 2-benzothiazolone-hydrazone hydrochloride (MBTH) and $FeCl_3$ to read the optical density at 650 nm. The Glucosamine hydrochloride standard was also reacted with MBTH and $FeCl_3$ for the colour development and O.D. reading, for the comparison purpose.

Plant material analysis:- Root, stem and leaves were powdered separately in small grinder and also sieved by 0.2 mm sieve. From sieved material, the total nitrogen was estimated by the micro-Kjeldahl method. Potassium and phosphorus was estimated through dry ashing method. For phosphorus, the magnesium acetate was added in the plant material before ashing while for potassium the ashing was done directly. Further, the molybdenum blue method was used for phosphorus estimation and flame photometer for potassium reading. All these methods were followed as suggested by Allen (1974). The nitrogen and potassium analysis of the non-mycorrhizal plants grown at 0 and 0.1 phosphate levels were not done due to very small quantity of plant material. Soil analyses were done as mentioned in Chapter I.

Results

The chitin estimation in the form of glucosamine units

showed a more definite trend of intensity of mycorrhizal infection in comparison to percentage infection observed by root slide method. In both the cases the mycorrhizal infection decreased with the increasing concentrations of phosphate in soil (Fig. 3.1a).

The mycorrhizal induced enhanced growth was observed at lower to medium doses of phosphates, which failed to remain so at higher concentrations. The shoot and root length (Fig.3.2), the leaf numbers and the leaf area of the mycorrhizal plants (Fig. 3.3, a,c) showed a similar growth behaviour with the initial increase and then a general decreasing trend along the phosphate gradients. The diameter of the first internode also exhibited the similar pattern but at 3.0 and 5.0 g phosphate levels a little increase was observed wihch was not significant statistically (Fig. 3.3, b).

However, when compared with the non-mycorrhizal plants the shoot length of the mycorrhizal plants was found significantly greater at all the phosphate levels except at the highest one while the root length increase could be observed to be significant only upto 0.4 g phosphate levels of soil. Similarly, the diameter of the first internode and the leaf number of the mycorrhizal plants were significantly greater than the non-mycorrhizal plants only at the lower levels of phosphate in soil. The leaf area of the mycorrhizal plants was always significantly greater than the non-mycorrhizal plants (Fig. 3.3 a).

Although the increasing trend in the root dry matter

production was observed in mycorrhizal as well as non-mycorrhizal plants but the rate of increase was reverse to each other along the phosphate gradient of the soil. The mycorrhizal plants produced significantly more root dry weight at lower levels of phosphate whereas non-mycorrhizal plants produced higher root dry weight at higher levels of phosphates and significantly higher at the highest phosphate level (Table 4.1). The shoot biomass and the total biomass produced by the mycorrhizal plants were significantly greater than the non-mycorrhizal plants at lower phosphate levels but due to greater shoot biomass production by the non-mycorrhizal plants at higher phosphate levels, the differences were reduced to a level not significant statistically (Table 4.1).

The root/shoot ratio was always lesser in the mycorrhizal plants than the non-mycorrhizal plants. The non-mycorrhizal plants gave a higher root/shoot ratio value at lower phosphate levels which became lower at intermediate doses of phosphate and again increased at highest phosphate levels (Table 4.1).

The nitrogen content was highest in the leaf than in the stem and root. The leaf nitrogen content of the mycorrhizal plants was highest at the 0 phosphate level and lowest at the highest phosphate level and almost constant with little decreasing trend at intermediate levels. On the other hand, the leaf nitrogen content of the non-mycorrhizal plants was higher compared to mycorrhizal plants upto 0.6 g phosphate levels only,

which declined further and showed a decreasing trend (Fig.3.4c). Almost similar trend was noted in stem and root of the non-mycorrhizal plants, but in case of mycorrhizal ones a constant level of nitrogen was maintained at all the phosphate levels of soil (Fig. 3.4 a,b).

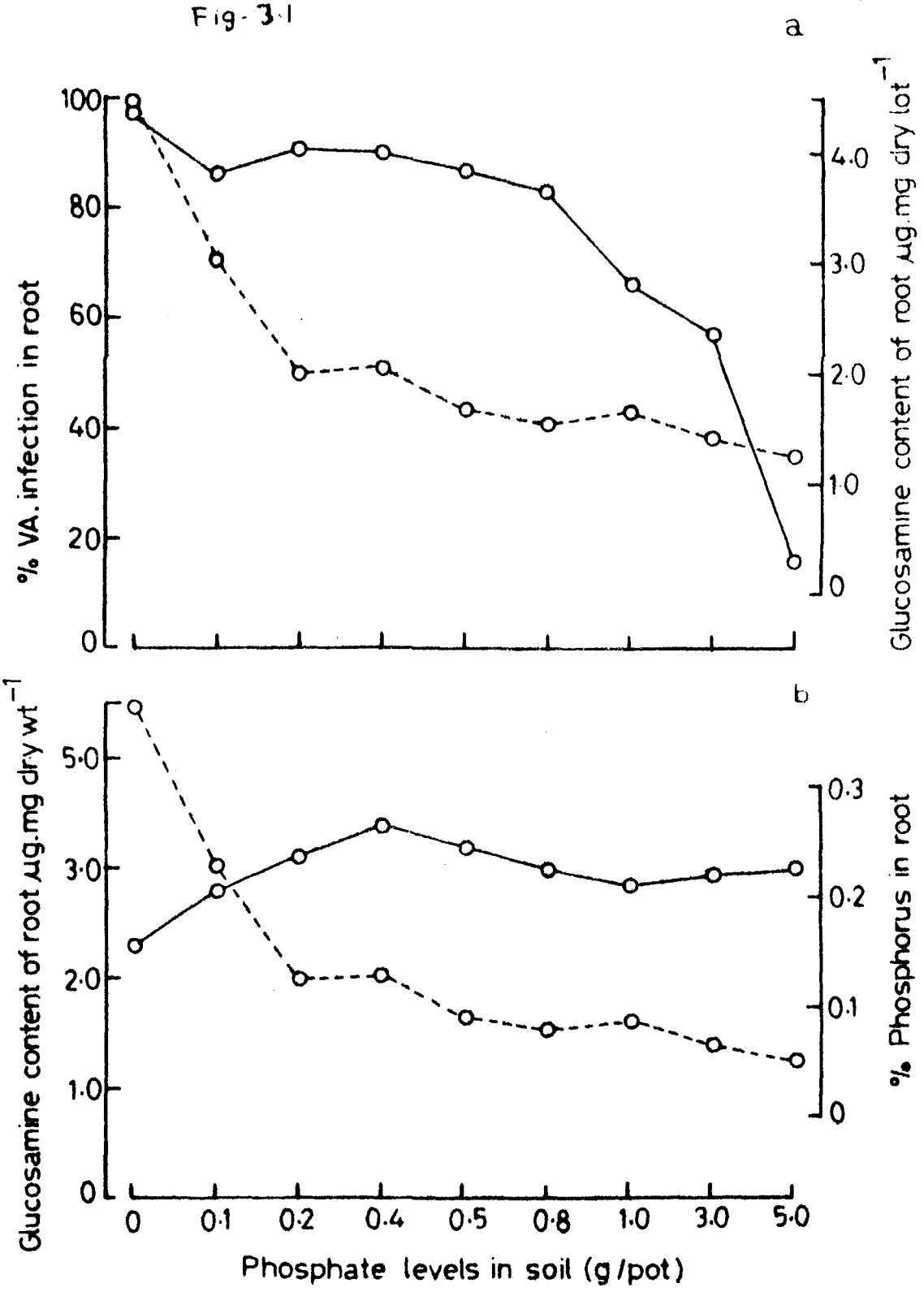
The potassium content in the leaf of non-mycorrhizal plant was higher upto 0.6 g phosphate levels which became less afterwards. The same in case of mycorrhizal plants was constant at all the phosphate levels (Fig. 3.4 f). In case of stem, the non-mycorrhizal plants had always higher potassium content than the mycorrhizal plants (Fig. 3.4 e). There was no difference in the potassium content in the root of either mycorrhizal or non-mycorrhizal plant and it exhibited a constant value without being affected by phosphate in soil (Fig. 3.4 d).

The mycorrhizal association had stimulating effect on phosphate uptake and a fairly high concentration of phosphate was observed in the leaf, stem and root of the mycorrhizal plants compared to other two i.e. Nitrogen and Potassium (Fig. 3.5). The mycorrhizal induced phosphate uptake was effective only at very lower level of phosphate in the soil. However, a constant high concentration of phosphate was maintained in the leaf, stem as well as root of the mycorrhizal plants, with a very little decreasing trend towards higher soil phosphate. On theother hand a continuous increasing trend of phosphate was observed in the leaf, stem and root of non-mycorrhizal plants along the increasing phosphate of soil.

Fig. 3.1a The relationship between the amount of glucosamine and the percentage VA-mycorrhizal infection in the root of the mycorrhizal plants of *E. adenophorum* grown in a range of phosphate additions in soil. O——O, percentage VAM infection; O----O, glucosamine content.

b The relationship between the amount of glucosamine and the phosphorus percentage in the root of the mycorrhizal plants grown in a range of phosphate additions in soil. O——O, percentage phosphorus in root; O----O, glucosamine content.

Fig-3.1


a
% VA. infection in root
Glucosamine content of root µg.mg dry lot⁻¹
100
80
60
40
20
0
4.0
3.0
2.0
1.0
0
b
Glucosamine content of root µg.mg dry wt⁻¹
% Phosphorus in root
5.0
3.0
2.0
1.0
0
0.3
0.2
0.1
0
0
0.1
0.2
0.4
0.5
0.8
1.0
3.0
5.0
Phosphate levels in soil (g/pot)

3.2 The root and shoot length of the mycorrhizal and non-mycorrhizal plants grown in a range of phosphate additions in soil.

Fig. 3.2

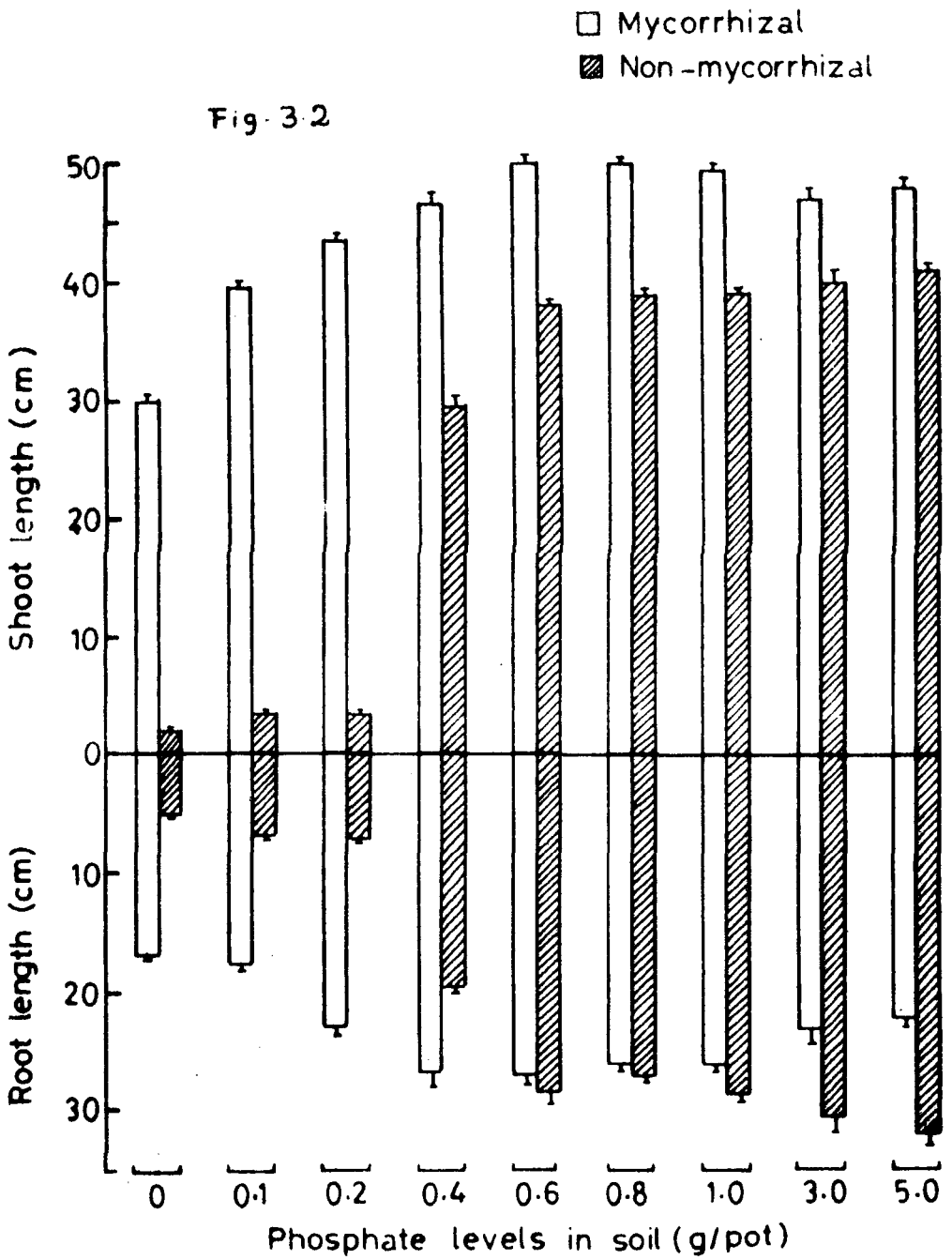

Mycorrhizal
Non-mycorrhizal
Shoot length (cm)
Root length (cm)
50
40
30
20
10
0
10
20
30
0
0·1
0·2
0·4
0·6
0·8
1·0
3·0
5·0
Phosphate levels in soil (g/pot)

Fig. 3.3 The differences in leaf area (a), stem diameter (b) and leaf number (c) of the mycorrhizal and non-mycorrhizal plants grown in a range of phosphate additions in soil.

Fig- 33

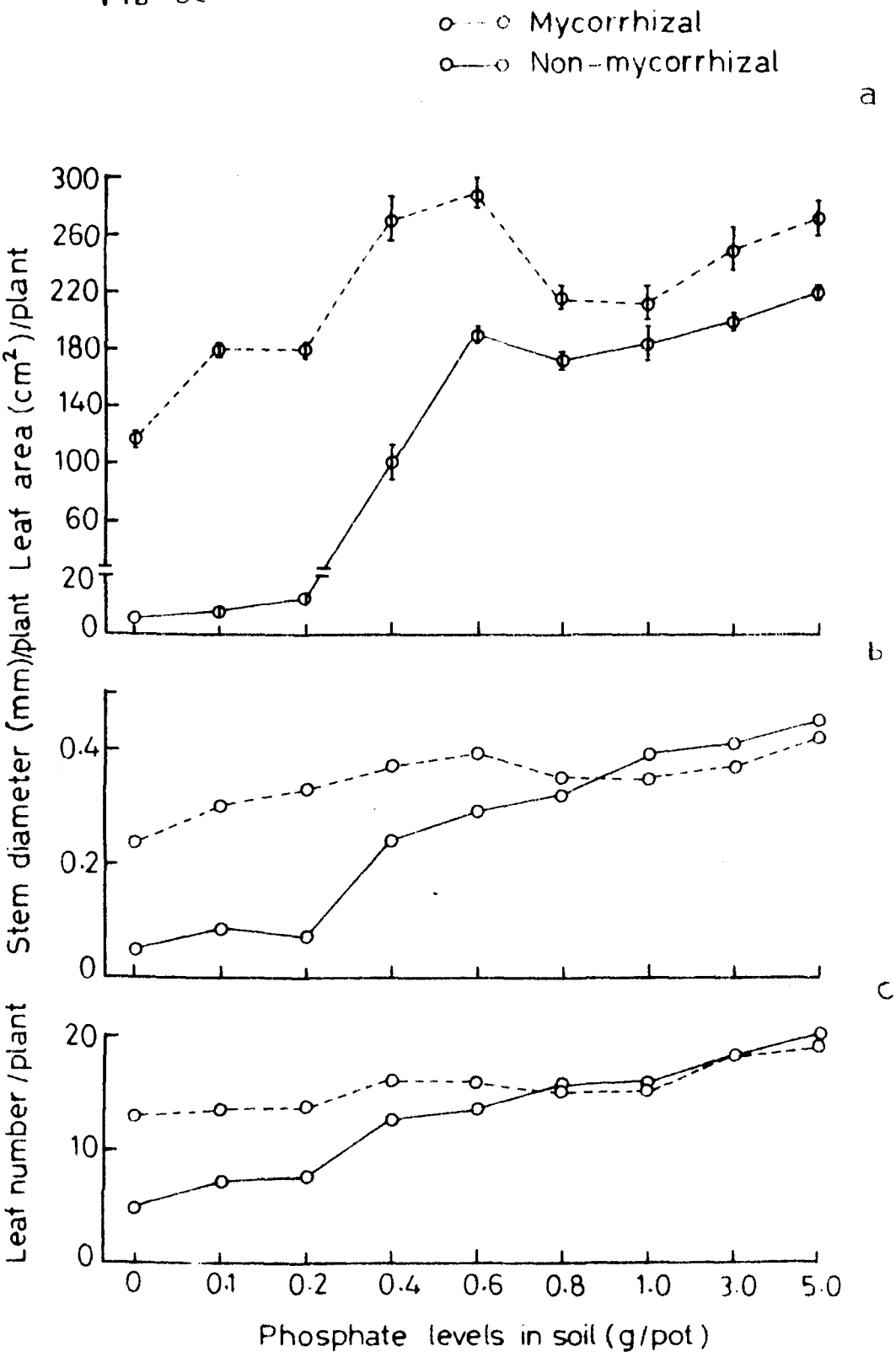

Mycorrhizal
Non-mycorrhizal
a
b
c
Leaf area (cm²)/plant
Stem diameter (mm)/plant
Leaf number /plant
300
260
220
180
140
100
60
20
0
0.4
0.2
0
20
10
0
0
0.1
0.2
0.4
0.6
0.8
1.0
3.0
5.0
Phosphate levels in soil (g/pot)

Fig. 3.4 The allocation of nitrogen and potassium in the leaf, stem and root of the mycorrhizal and non-mycorrhizal plants grown in a range of phosphate additions in soil.

Fig-3.4

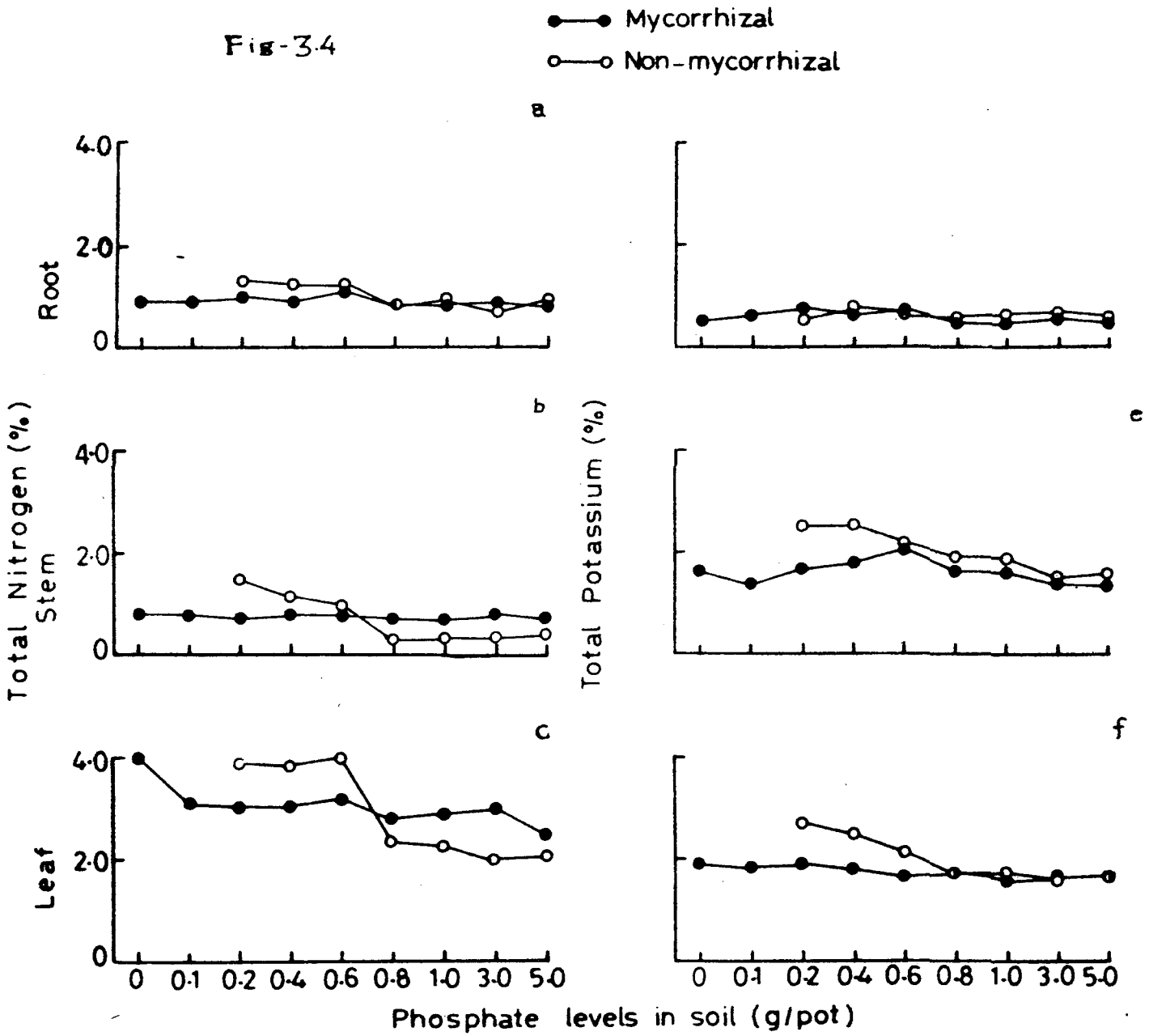

Mycorrhizal
Non-mycorrhizal
a
b
c
d
e
f
Root
Stem
Leaf
Total Nitrogen (%)
Total Potassium (%)
4·0
2·0
0
0 0·1 0·2 0·4 0·6 0·8 1·0 3·0 5·0
Phosphate levels in soil (g/pot)

Fig. 3.5 The allocation of phosphate in the leaf, stem and root of the mycorrhizal and non-mycorrhizal plants grown in a range of phosphate additions in soil.

Fig 3.5

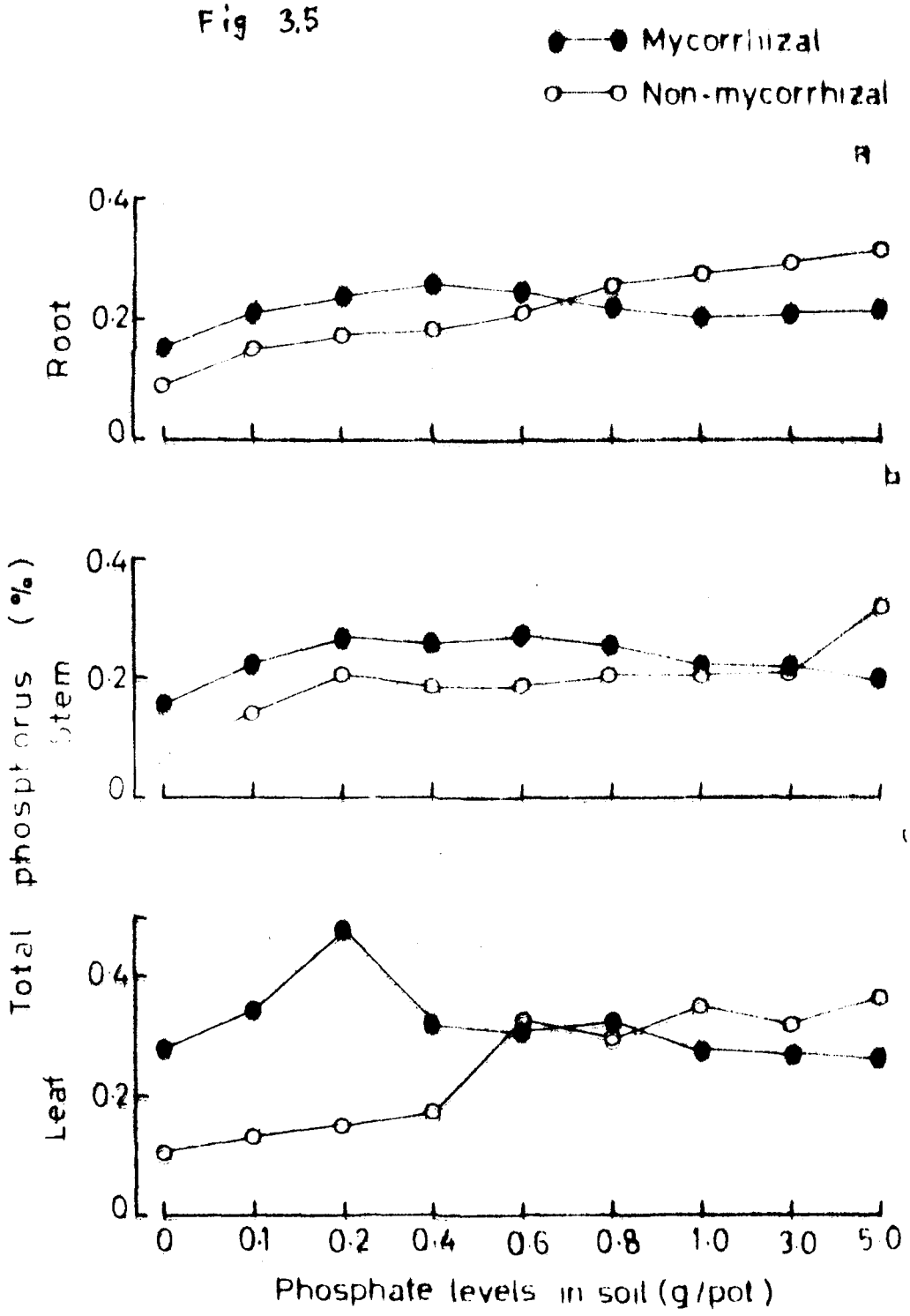

Mycorrhizal
Non-mycorrhizal
a
b
c
Root
Stem
Leaf
Total phosphorus (%)
0·4
0·2
0
0
0·1
0·2
0·4
0·6
0·8
1·0
3·0
5·0
Phosphate levels in soil (g/pot)

Table: 4.1 Root wt, shoot wt, total biomass and root/shoot ratio of the mycorrhizal plants grown in a range of phosphate levels on per plant basis.

Phosphate levels g/pot		Root wt (g)	Shoot wt (g)	Total Biomass (g)	Root/shoot ratio
0	+M	0.09±0.04**	0.41±0.12	0.50±0.16**	0.24±0.084**
	=M	0.0029±0.0007	0.005±0.0008	0.008±0.001	0.57±0.18
0.1	+M	0.15±0.07**	0.74±0.17**	0.9±0.23**	0.2±0.05*
	-M	0.005±0.003	0.009±0.004	0.01±0.004	0.46±0.22
0.2	+M	0.20±0.05**	0.76±0.2**	0.96±0.24**	0.27±0.07
	-M	0.004±0.003	0.011±0.006	0.015±0.007	0.38±0.28
0.4	+M	0.21±0.05	0.98±0.4*	1.24±0.4*	0.24±0.09
	-M	0.11±0.6	0.46±0.3	0.60±0.41	0.3±0.144
0.6	+M	0.21±0.04	1.2±0.23**	1.42±0.27**	0.18±0.03*
	-M	0.186±0.07	0.68±0.11	0.86±0.14	0.28±0.11
0.8	+M	0.22±0.04	0.93±0.17	1.15±0.19*	0.27±0.06
	-M	0.20±0.07	0.66±0.14	0.86±0.19	0.30±0.11
1.0	+M	0.21±0.06	0.99±0.3	1.2±0.34	0.22±0.03
	-M	0.24±0.07	0.73±0.3	0.97±0.36	0.28±0.02
3.0	+M	0.24±0.13	1.13±0.45	1.37±0.56	0.2±0.04*
	-M	0.28±0.11	0.86±0.18	1.14±0.26	0.32±0.09
5.0	+M	0.28±0.09*	1.28±0.39	1.56±0.44	0.23±0.1*
	-M	0.46±0.18	1.17±0.28	1.58±0.36	0.41±0.12

Values are the mean of 10 reolicates with standard deviation.

** Significant at 1% level, * Significant at 5% level.

Discussion

The estimation of mycorrhizal infection by chitin digestion method was found comparatively more suitable than the observation of the percentage infection by root slide method only. The percentage infection was positively correlated with the glucosamine content of the mycorrhizal root and the correlation coefficient value (0.601) was very near to the statistical significant value (0.632) at 5 percent level. Becker and Gerdemann (1977) also found the similar relationship between percentage infection and the glucosamine content of the mycorrhizal root. But at the highest soil phosphate level, when the percentage infection of the root was measurably less, the glucosamine content was not truely as such (Fig. 3.1 a). This observation put some doubt on the sensitivity of the chitin assay technique, in case of least infected root materials. Similar doubts have been raised by Tatareu and Touze (1975), Hepper (1977), Sakurai et al., (1977) and Sharma et al., (1977), on the plea that the age of the fungal mycelium, the different structural pattern of the mycorrhizal fungus within the root tissues, and the different environmental conditions may play significant role in the composition of fungal chitin. Donald and Mirocha (1977) and Nandi (1978) suggested to apply chitin assay technique only when the presence or absence of the infection is to be determined. The chitin assay would be more perfect if added with the chemical analysis of the fungal compounds and other histological observations (Whipps and Lewis, 1980). However, the result of this experiment is in agreement with Hepper (1977)

who suggested to use the chitin assay technique in combination with the stained root slide observation.

The root/shoot ratio in the mycorrhizal plant was always less, indicating their proportionate growth at all the phosphate levels, whereas the same in case of non-mycorrhizal plant was very high at the lower phosphate levels, which gradually became less. However, if considering the lower soil phosphate stage only, which was the real phosphate deficient condition and subsequently, the congenial condition for the mycorrhizal effect, the root/shoot ratio value of the mycorrhizal plant was significantly lesser than the non-mycorrhizal ones. The lesser root/shoot ratio in the mycorrhizal plants has also been reported by Hayman and Mosse (1971), Mosse and Hayman (1971), and Becker and Gerdemann (1977).

The percentage nitrogen in the leaf, stem and root of mycorrhizal plants was more than non-mycorrhizal plants at higher phosphate level but less at lower phosophate level. Though, not with the mycorrhizal experiments, Williams (1948) and Hills et al., (1970) found an increase in the nitrogen uptake in the plants grown in a grade of phosphates, but no report on the mycorrhizal induced nitrogen uptake have been published except in leguminous plants (Gerdemann, 1975).

The explanation for the lower potassium content in the plant tissues of the mycorrhizal plants, even at the active phase of the mycorrhizal infection, i e. at the lower phosphate level in soil, would be difficult, except to assume the dilution

effect due to large volume of the plant tissues of the mycorrhizal plants, as has been discussed by Menge et al., (1978c) in case of citrus plants. In the highly infertile soil the probable uptake of the potassium through the large volume of soil exploration by the mycelial extension of the mycorrhizal fungi has also been pointed out by Mosse (1973a) and a similar view point may be extended to the nitrogen uptake.

The percentage infection or the glucosamine content of the mycorrhizal root was inversely proportional to the amount of phosphate in soil. The root length of the mycorrhizal plants also behaved according to the glucosamine content of the roots, but the phosphate content of the mycorrhizal root was negatively correlated with the fungal chitin (Fig. 3.1b). These observations were similar to the findings of Sanders (1975) and Menge et al., (1978a), who found that the high phosphate concentration within the root system regulated the infection and colonization of mycorrhizal fungi and not the phosphate concentration of the soil.

The general growth superiority of the mycorrhizal plants over non-mycorrhizal plants at lower soil phosphate level seemed to be the direct effect of the mycorrhizal induced increased growth of the former, but at the higher doses of the phosphate the growth of the mycorrhizal as well as non-mycorrhizal plants was almost similar as found by Pairunan et al.,(1980) The reduction in the mycorrhizal induced growth, when the phosphate in soil is no longer a limiting factor, has also been

reported by Daft and Nicolson (1966), Pairunan et al., (1980).

It appeared that the stimulating growth influence of the mycorrhizal infection was less in case of root growth than the shoot, because the point of no significant difference between the root growth of the mycorrhizal and non-mycorrhizal plants was reached at very lower phosphate level (after 0.2 g/pot level), while the same in the shoot growth was reached at comparatively higher phosphate levels (after 0.6 g/pot level). The probable reason for this might be the soluble nature of the phosphate which could be absorbed by the non-mycorrhizal plants also and the roots being in the direct contact of the source would have responded earlier to the shoot growth.

The higher amount of phosphate in the leaf, stem and root of the mycorrhizal plants at lower levels of phosphate was due to mycorrhizal infection, which declined at higher phosphate levels probably due to reduction in mycorrhizal action. But compared to the other two nutrients i.e. Nitrogen and Potassium, the accumulation of phosphate was fairly higher in the leaf, stem as well as root, which may be attributed to the higher concentration of the soluble phosphate in the soil. According to Epstein (1972), if the nutrients are in excess, they are accumulated within the plant tissues above the levels that immediately promote growth. The high phosphate concentration in the roots of the mycorrhizal plants may have some regulating mechanism in the phosphate flow to shoot (Allen, 1981). The various ways, through which the mycorrhizal fungi extract

phosphate from soil has been well discussed by Allen et al. (1981).

The higher concentration of the nitrogen, phosphorus and potassium in the leaf, than the root and stem may be attributed to the preferential demanding sites, explained as the point of greatest meristematic activity or "sink-strength" by Chapin (1980).

Although the percentage nitrogen and potassium was lesser in the mycorrhizal plants at lower levels of phosphate, but it never declined abruptly at any levels of phsphate, as was exhibited by the non-mycorrhizal plants. Therefore, it may be concluded that whether mycorrhizae helps in the uptake of nitrogen and potassium or not, is open to question, but certainly it helps to retain or maintain a definite status of nutrients within the plant tissues without being affected by the highly imbalanced nutrient condition of the soil which was created by the higher phosphates, in the present experiment. Further, the exceptionally greater growth performance of the Eupatorium adenophorum, inoculated with the Glomus tenuis, than the non-mycorrhizal plants, even without any addition of phosphate i.e. at 0 level, in soil, it appeared that the Eupatorium adenophorum is a highly mycorrhizal weed species and, this also may be one of the reasons as to why it grows luxuriantly in the phosphate deficient soil of the North-East India.

The effect of indigenous and introduced vesicular-arbuscular endophytes on the growth and nutrient uptake of *Eupatorium riparium* Regel.

Introduction

The different strains of vesicular-arbuscular mycorrhizae differ in their effect on the growth and nutrient uptake of the plants, particularly the phosphate (Mosse, 1972b; Powell, 1975b, 1979b; Hall, 1976). The introduced endophytes have been reported to be superior to the indigenous ones in promoting the growth (Mosse and Hayman, 1971; Mosse, 1975,1977). The stimulated growth of the pre-inoculated seedlings, planted in the test soil, under glass house conditions (Mosse and Hayman, 1971; Mosse *et al*., 1976; Mosse, 1977; and Powell and Daniel, 1978) and in the field soils (Khan, 1972, 1973; Saif and Khan, 1977) have been shown. Even the two isolates of the same *Endogone* species have been found to differ in their ability to enhance the growth (Abbott and Robson, 1978). However, the effect of the combined treatment of the two endophytes in the sterilized soil, on the infection and colonization of the root and on the growth and nutrient uptake of the plants has not been studied. In the present study the effect of the introduced endophytes *Glomus fasciculatus* and *Glomus mosseae* in comparison to the indigenous endophyte *Glomus tenuis* and the combination of the indigenous endophyte with the two introduced ones, has been investigated with respect to root colonizing ability, the growth stimulation and the nutrient uptake.

Materials and Methods

The garden soil with the soil properties as follows: pH 5.5, organic matter 2.87 (%), total nitrogen 0.14 (%), available phosphorus 4.3 (ppm) and exchangeable potassium 0.17 (mg/g) was mixed with the sand in 1:1 ratio, and sterilized in the autoclave. 3 Kg of this soil mixture was filled in 30 plastic pots and left for 15 days under moist condition to regain the usual microbial activity. The mycorrhizal inoculum used,was the Glomus fasciculatus, (provided by Dr Menge, USA). G. mosseae (provided by Dr. Hayman, U.K.) and the indigenous ones Glomus tenuis (isolated locally) which were maintained in pure pot culture on the host Eupatorium adenophorum Spreng. 50 ml of the roots and soil of the above mentioned three mycorrhizal strains were evenly spread 3 cm. deep in the soils in 15 pots, using 5 pots for each, separately. In another set, the mixture of the indigenous endophyte G. tenuis + G. fasciculatus, and G. tenuis + G. mosseae, (25 ml each in both) was used as inoculum. A control of non-mycorrhizal inoculation was kept for the comparison sake.

The seeds of Eupatorium riparium were germinated in a moist chamber at $30\pm2^{o}C$ in a B.O.D. incubator and seven days old seedlings were transplanted in each pots. After another 7 days of growth a thinning was done in order to allow only two healthy seedlings per pot to grow further. 10 replicates of plants were maintained for each of the treatment till the end of the experiment. The experiment was conducted under glass house conditions between April and October, 1981 and the

harvesting was done after 22 weeks. The plants were watered with tap water, every alternate day for 4 weeks in the beginning and thrice a week afterwards.

The dry weight of the root and shoot was determined by drying them at 80°C in oven for 48 hours, and the nutrient analysis of the plant material as well as the soil was done according to the methods described in chapter 1 and 4.

The infection percentage was estimated by the root clearing and staining method (Phillips and Hayman, 1970). The measurement of the intensity of infection was based on the visual observation in which the root segments were grouped into following frequency classes i.e. 0-25% = +, 25-50% = ++, 50-75% = +++ and 75-100% = ++++. Similarly, the quantification of each of the mycorrhizal fungi, within the root cortex in the combined treatments was based on the visual observation with the limited purpose of describing their behaviour only.

Results

In the single treatments of mycorrhizal fungi, the indigenous endophyte G. tenuis produced the 100% infection and intensity and same was found in both the combined treatments (Table 5.1). However, the competitive colonizing ability of the two fungal symbionts was different in the two combined treatments. In one combination, the colonization of G. tenuis was highly suppressed by the other partner, i.e. G. fasciculatus while in another one, G. tenuis suppressed the

spread of G. mosseae. The proportion of the two mycorrhizal fungi was 1:4 (G. tenuis : G. fasciculatus) and 4:1 (G. tenuis : G. mosseae) (Table 5.1). The infection percentage as well as the intensity of infection both were higher in G. fasciculatus than G. mosseae.

The growth of the plants did not show any definite trend in response to mycorrhizal infection nor was it related to the nutrients uptake. However, the highest infection levels were followed by higher phosphate concentration in the root (Fig. 4.2).

The G. fasciculatus superceded all the other mycorrhizal strains in promoting the growth of root as well as shoot, while the local isolate G. tenuis exhibited the poorest growth performance. Stunted root growth was observed in the treatment of G. tenuis which was also reflected on shoot growth (Fig. 4.1). The combination of G. tenuis with G. mosseae promoted the shoot growth but retarded the same in combination with G. fasciculatus, when compared to the single treatment of G. mosseae and G. fasciculatus respectively, but the root growth was reduced in both the cases (Fig. 4.1).

In general, all the mycorrhizal treatments were superior to the non-mycorrhizal control in promoting the growth of the plants. Among the treatments, the effectiveness of the different VAM fungi in the enhanced growth of the shoot was in the following decreasing order: G. fasciculatus > G. fasciculatus + G. tenuis > G. mosseae + G. tenuis > G.

mosseae and G. tenuis, while in case of root the trend was: G. fasciculatus > G. mosseae > G. mosseae + G. tenuis > G. fasciculatus + G. tenuis > G. tenuis (Fig. 4.1). Whereas G. fasciculatus showed the significantly greater growth performance, G. tenuis showed the significantly lesser growth in all the sets. But as far as total biomass is concerned, the treatment of G. mosseae and both the combined treatments had almost similar effect which showed significantly greater growth performance from G. tenuis as well as from control but significantly lesser from G. fasciculatus (Fig. 4.1).

The root/shoot ratio was highest (0.84) in control and least (0.18) in the combined treatment of G. fasciculatus + G. tenuis, but compared to control the root/shoot ratio value was significantly lower in all the mycorrhizal treatments (Fig. 4.2).

The nutrient analysis of the plant material showed the highest concentrations of nitrogen in the shoot of the plants treated with G. mosseae followed by G. mosseae + G. tenuis, but in the rest of the treatments it was not different from control (Fig. 4.3).

The phosphorus uptake was stimulated by all the mycorrhizal treatments which was evident from the comparison from control (Fig. 4). The most effective mycorrhizal fungi for the phosphorus uptake in shoot was G. mosseae followed by G. tenuis and G. fasciculatus. The combination of G. tenuis either with G. fasciculatus or with G. mosseae reduced the

uptake of phosphorus in shoot, compared to the single treatment of G. fasciculatus and G. mosseae respectively (Fig. 4.3).

It appeared that the G. mosseae either alone or in combination with G. tenuis favoured the potassium uptake more than control but in other mycorrhizal treatments the potassium concentration in the shoot was not different from control except G. fasciculatus + G. tenuis. The percentage potassium content of the shoot was highest in combined treatment of G. mosseae + G. tenuis.

The allocation pattern of the nutrients in root did not show any definite trend but the dual infection of G. mosseae + G. tenuis and G. fasciculatus + G. tenuis favoured the higher allocation of the nutrients towards root, compared to others (Fig. 4.3).

Discussion

The growth of the plants was not related to the infection or the intensity of infection of the roots by either of the mycorrhizal treatments and according to Mosse (1972a), it is not necessary that the effectivity of the mycorrhizal species should be related to the state of infection. The introduced endophytes increased the growth more than the indigenous species G. tenuis, even at the lower infection levels. This observation was similar to others (Powell, 1976; Mosse, 1977), who also found that the introduced endophytes did increase

Fig. 4.1 The effect of different VA-mycorrhizal strains on the root, shoot and total biomass production in *E. riparium*.

Fig 4.1

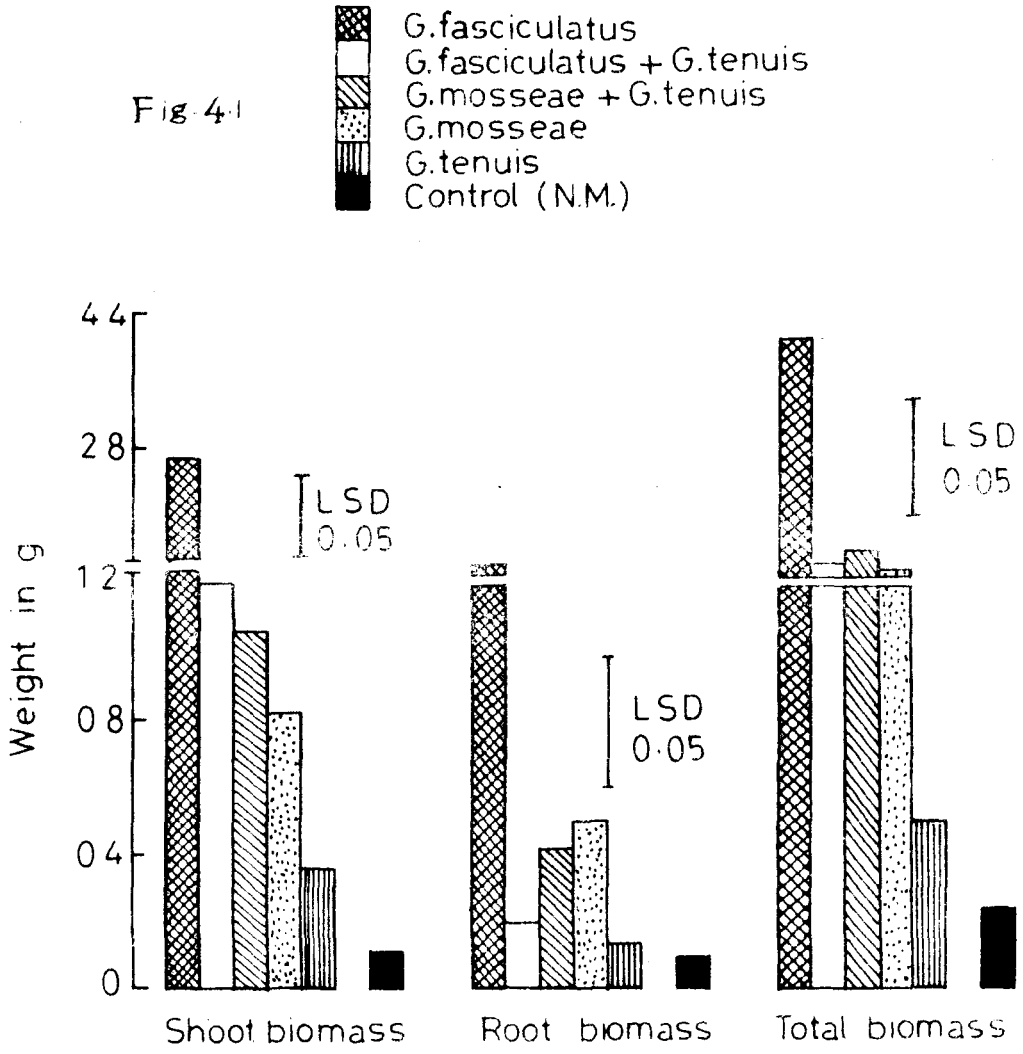

G.fasciculatus
G.fasciculatus + G.tenuis
G.mosseae + G.tenuis
G.mosseae
G.tenuis
Control (N.M.)
Weight in g
4 4
2 8
1 2
0 8
0 4
0
LSD 0.05
LSD 0.05
LSD 0.05
Shoot biomass
Root biomass
Total biomass

Fig. 4.2a The root/shoot ratio of the plants inoculated with the different mycorrhizal endophytes.

b The relationship between the percentage VA-mycorrhizal infection and the percentage phosphate content of the root under the influence of different mycorrhizal endophytes. Histograms , mycorrhizal infection; O——O, root phosphate percentage.
G.m.,*Glomus mosseae*; G.f., *G. fasciculatus*; G.m.+ G.t., *G. mosseae* + *G. tenuis*; G.t., *G. tenuis*; G.f. + G.t., *G. fasciculatus* + *G. tenuis*; Cont., control (non-mycorrhizal).

Fig-42

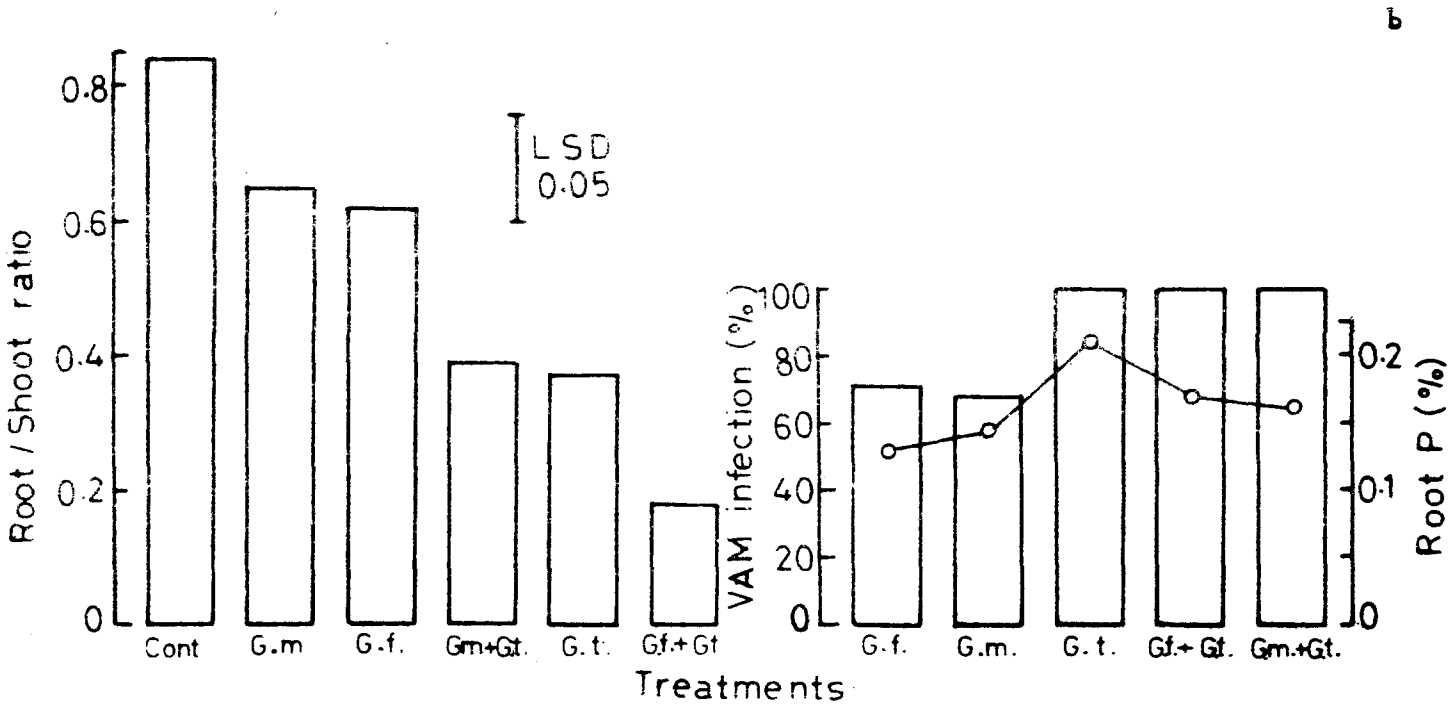

b
Root / Shoot ratio
0.8
0.6
0.4
0.2
0
LSD
0.05
Cont
G.m
G.f.
Gm+Gt.
G.t.
Gf.+Gt
VAM infection (%)
100
80
60
40
20
0
G.f.
G.m.
G.t.
Gf.+ Gt.
Gm.+Gt.
0.2
0.1
0
Root P (%)
Treatments

Fig. 4.3 The allocation of nitrogen phosphorus and potassium in the root and shoot of the plants inoculated with different strains of VA-mycorrhizal fungi. Abbreviations are same as in Fig. 4.2. NM, non-mycorrhizal.

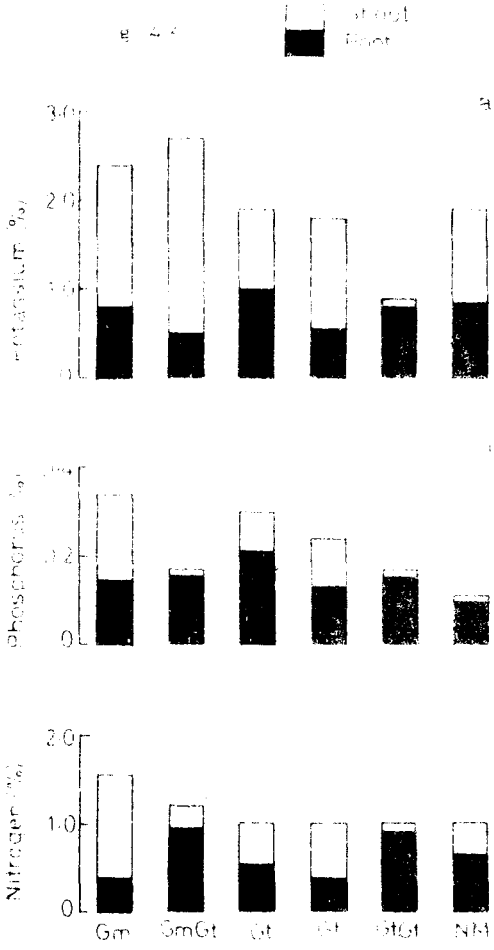

2.0
1.0
0
Gm
GmGt
Gt
StGt
NM

Table: 5.1 The percentage infection ahd the intensity of infection of different VAM fungi.

VAM species	% infection	Intensity
Glomus fasciculatus	71	+++
Glomus fasciculatus + Glomus tenuis	100 (4:1)	++++
Glomus mosseae	68	++
Glomus moasese + Glomus tenuis	100 (1:4)	++++
Glomus tenuis	100	++++

the growth without showing increase in percentage infection. Moreover, the achievement of high percentage infection may also be delayed if the root growth is fast (Sutton, 1974).

The exceptionally poor growth performance by G. tenuis which was a native endophyte of E. riparium was surprising. However, the behaviour of a particular mycorrhizal fungi in the field cannot be expected to be the same under glass house conditions (Powell and Daniel, 1978, discussion part). The growth depression in grass species inoculated with G. tenuis under glass house conditions was noticed by Crush (1973). G. tenuis in combination with G. fasciculatus as well as G. mosseae adversely affected the root growth compared to G. fasciculatus and G. mosseae separately. The double endophytic treatment was also followed by the high root phosphate concentration almost equal to shoot phosphate concentration. The greater uptake of phosphate by G. tenuis is well known (Powell and Daniel, 1978) and probably same source of P is utilised by all mycorrgizal fungi (Powell, 1975b). Therefore, the possibility of uptake of P by the double endophyte at an enhanced rate may be presumed which ultimately should be attributed to the very high P concentration in the root.

The arrested growth of the roots in case of G. tenuis and its combination with other two may be explained in term of phosphate induced toxicity in root (Mosse, 1973b). The retention of phosphate in root, which is near to source, may be at the cost of P supply in the shoot (Brouwer, 1962) and this may also play some role in controlling the P concen-

tration in shoot (Smith, 1982). Therefore, the extremely reduced growth of the root due to high concentration of P, achieved through the efficient mycorrhizal activity of G. tenuis, may be presumed to be one of the cause of reduced growth even at 100% infection level. It would be more clear if it is assumed that these phenomena are interrelated and interregulatory.

It is not possible in this experiment to explain the exact mechanism of the mycorrhizal species, which stimulated the growth of the plant without showing any relationship with the percentage infection and percentage nutrient concentration. But whatever, mechanism may be there, decidedly G. fasciculatus was much more efficient than others in promoting the growth of E. riparium. Even in combination with G. tenuis, it suppressed the spread of G. tenuis in root cortex and the total biomass produced by this combined treatment was almost equal to the biomass produced by G. mosseae and G. mosseae + G. tenuis and all were significantly greater than the indigenous endophyte G. tenuis as well as the control. The high efficiency of mycorrhizal strain E_3 (Gilmore and Fish, 1968) which is not other than G. fasciculatus (Hall 1979) in acid soils has also been observed by Mosse (1972a), and Powell (1976). Mosse (1972a) also indicated the inefficiency of G. mosseae in acid soil but the combination of G. mosseae and G. tenuis had slightly stimulating effect on the shoot growth compared to G. mosseae alone. Although, G. tenuis suppressed the colonization of G. mosseae in the combined treatment,

the dual infection had favourable effect on shoot growth. Mosse (1972a) expressed the view that the contaminating micro-organisms associated with different mycorrhizal endophytes can also have some effect on the growth of the host. The rhizosphere organisms are well known to compete with plants for nutrients especially P (Barber and Loughman, 1967; Barber, 1968; Bowen and Rovira, 1966). The changed role of endo-mycorrhizal fungi G. tenuis, as a typical rhizosphere organism under different environments has also been suggested by Crush (1973). It may be possible that G. tenuis which is competent enough to function in acidic environments, would have created a congenial atmosphere in soil for stimulated activity of G. mosseae which subsequently resulted into increased shoot growth.

The root/shoot ratio was highest and significantly greater in control than any other mycorrhizal treatments, which was a common observation of others (Mosse, 1973a, Gurdemann, 1975). However, the rest of the mycorrhizal treatments showed different values for root/shoot ratio which may be due to differences in efficiency of mycorrhizal fungi and the biomass allocation strategy of the host in response to endophytes.

The nitrogen concentration in shoot slightly increased in case of G. mosseae singly and in combination with G. tenuis and same was the pattern with potassium concentration, but in no case it was lesser than control except the potassium

concentration in case of G. fasciculatus + G. tenuis. In general, the root N P K concentration was highest in both the combined treatments and in G. tenuis. The involvement of G. tenuis in higher phosphate uptake has already been discussed earlier, but regarding other nutrients also, this may hold good, because the uptake of other nutrients may be indirectly linked with the increased uptake of phosphate through the mycorrhizal chanel (Smith et al., 1981). It cannot be concluded, but it appeared that G. mosseae had sone effect on the potassium uptake which was found to be further higher in combination with G. tenuis. The role of mycorrhiza in potassium uptake has also been indicated by Mosse (1973a) and Powell (1975c).

CHAPTER VI

The effect of vesicular-arbuscular mycorrhizae on the growth of Osbeckia crinita Wall. ex. D.Don. grown in sterilized and unsterilized natural soil.

Introduction

The effectiveness of the vesicular-arbuscular mycorrhizae under different soil conditions have been studied (Mosse, 1973a). The artificial inoculation has comparatively less effect on the growth of the plants than those with the natural inoculum (Smith & Smith, 1981b). The growth of plant is very much influenced by the associated microorganisms in the soil. The sterilization of soil in order to remove the existing mycorrhizal propagules also removes the natural microbial population from the soil, which may be both benificial or harmful. Therefore, the comparative study on the effect of VA-mycorrhizae was undertaken to observe the behaviour of the mycorrhizae on the successive stages of growth of Osbeckia crinita.

Materials and Methods

The field soil, where the natural O. crinita population was growing was collected from upper 0-10 cm depth and sieved through Mesh. No. 30 (pore size 500 u). The soil was sandy in nature with following properties: pH 5.3, organic matter 4.75 (%), total nitrogen 0.30 (%), available phosphorus 3.86 (ppm) and exchangeable potassium 0.14 (mg/g). The total

Endogone spore population was 46/g fresh weight of soil.

A portion of the soil was sterilized in an autoclave and the sterilized soil was filled in 30 plastic pots of 1.5 Kg capacity. 15 pots from this lot were used for the mycorrhizal inoculation and the same number was used for the non-mycorrhizal control experiment. The natural unsterilized soil was also filled in another 15 pots for the natural mycorrhizal experiment. 5 g of the sieved soil in the form of inoculum was mixed thoroughly to the upper 3 cm soil layer of the pots meant for the inoculated mycorrhizal experiment and Endogone free, filtered washings of the same soil was mixed in the pots to be used for the control experiment.

Seeds of Osbeckia crinita were kept in an incubator at 30±2°C for 24 hours under moist condition and just burst seeds were transferred to the pots at the rate of 6 seeds per pot. After 15 days of growth, the seedlings were thinned to 2 per pot with a care of allowing only healthy and alike seedlings to grow further in all the treatments.

The experiment was conducted under glass house conditions during the months March-August 1981 and were watered thrice a week with ordinary tap water. Three harvests were taken at an interval of 2 months and 5 pots with 10 plants altogether were taken as replicates.

Measurements:- The percentage mycorrhizal infection was measured by the root clearing and staining technique of Phillips

and Hayman (1970). The plant materials were analysed for nitrogen, phosphorus and potassium, in the last harvest only. The soil analysis as well as the plant material analysis was done according to the methods described in chapters 1 and 4. The dry weight of the root and shoot was determined by drying at 80°C for 48 hours and reweighing till constant weight.

Results

Mycorrhizal infection:- The mycorrhizal infection in the inoculated plants increased at second harvest but again decreased at third harvest while the vesicles formation could increase only at last harvest. The intensity of infection showed a increasing trend in inoculated plants (Table 6.1). The infection percentage and the intensity of infection in case of natural mycorrhizal plants were high throughout and no change in the occurrence of vesicles could be observed at any harvest (Table 6.1).

Growth:- The growth performance of the plants in both the mycorrhizal treatments was significantly greater than the non-mycorrhizal control at all the stages of growth. At the time of first harvest, the natural mycorrhizal fungal association favoured the growth of the plants, over inoculated mycorrhizal counterparts but the results were statistically not significant. However, after initial decrease, the inoculated plants showed a continuous increase in the growth at other two successive harvests (Fig. 5.1). The shoot growth of the inoculated plants

was significantly greater than the natural mycorrhizal plants at second harvest and at the time of third harvest the root, shoot and the total biomass, all were significantly higher in the inoculated plants than the natural ones (Fig. 5.1). The root/shoot ratio in both the mycorrhizal treated plants was lesser than the control except at last harvest when a reverse trend was observed (Fig. 5.2).

Nutrient uptake:- The nitrogen content found in leaf, stem and root of both the type of mycorrhizal treated plants was not different from control sets (Fig. 5.3). The potassium also remained unchanged in the leaf and stem but was higher in the roots of the inoculated plants over natural mycorrhizal treatment and control. Among the mycorrhizal treatment the highest potassium percentage was observed in the roots of the inoculated pants (Fig. 5.3).

The phosphate content of the mycorrhizal plants was higher than the control at all the harvests. The amount of phosphate accumulated in the leaf of the inoculated plant was as high as in root while in the natural mycorrhizal plants the root phosphate content was higher than the leaf (Fig. 5.3).

Discussion

The higher infection percentage coupled with the very high intensity of infection observed in natural mycorrhizal treated plants (Table 6.1) was probably due to higher mycorrhizal inoculum density in the unsterilized natural soil than the

Fig. 5.1 The root, shoot and total biomass production by *Osbeckia crinita* growing in natural unsterilized soil, sterilized soil inoculated with mycorrhizal fungi and the sterilized soil without mycorrhiza at different stages of growth.

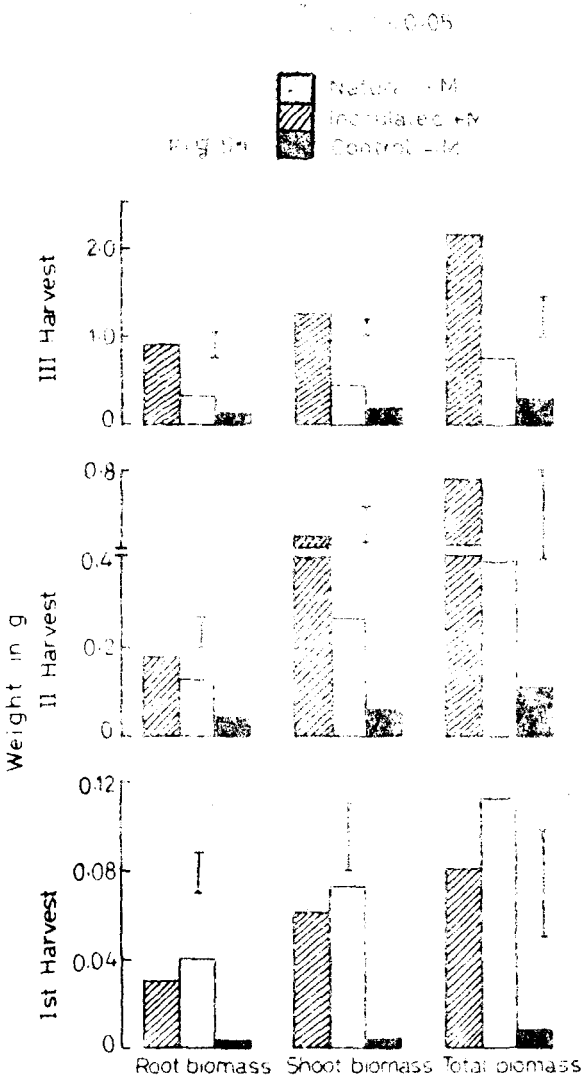

Weight in g
III Harvest
II Harvest
I st Harvest
2·0
1·0
0
0·8
0·4
0·2
0
0·12
0·08
0·04
0
Root biomass
Shoot biomass
Total biomass

Fig. 5.2 The root/shoot ratio of the plants growing in natural unsterilized soil, sterilized soil inoculated with mycorrhizal fungi and the sterilized soil without mycorrhiza at different stages of growth.

Fig- 5.2

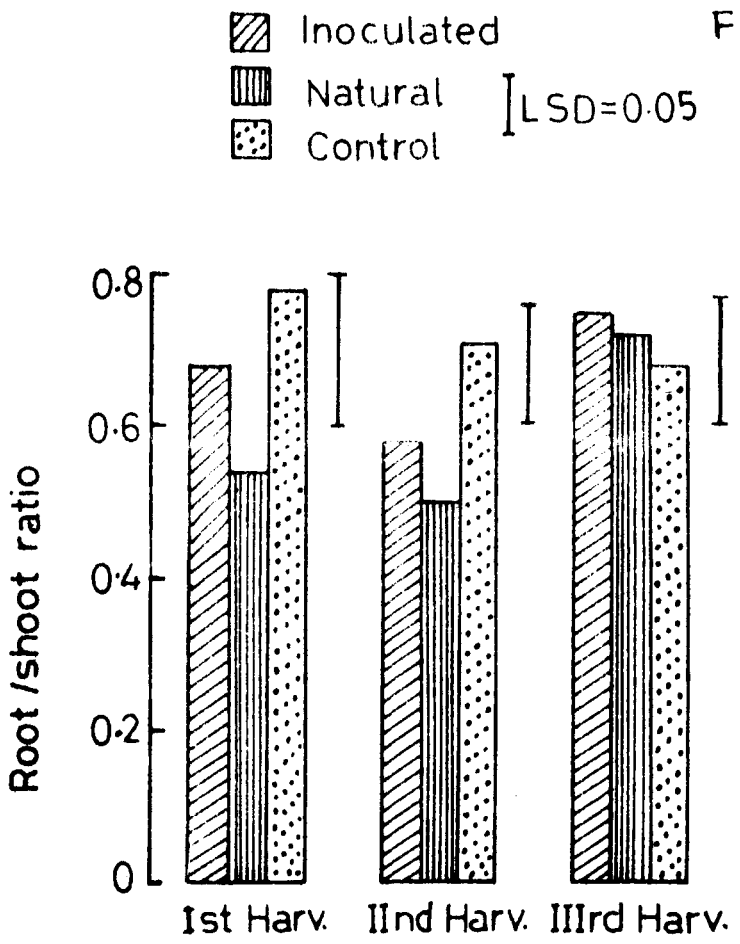

Inoculated
Natural
Control
LSD=0.05
Root /shoot ratio
0.8
0.6
0.4
0.2
0
Ist Harv.
IInd Harv.
IIIrd Harv.

Fig. 5.3 The allocation of nitrogen, phosphorus and potassium in the leaf, stem and root of *Osbeckia crinita* grown in natural unsterilized soil, sterilized soil inoculated with mycorrhizal fungi and the sterilized soil without mycorrhiza.

Fig 53

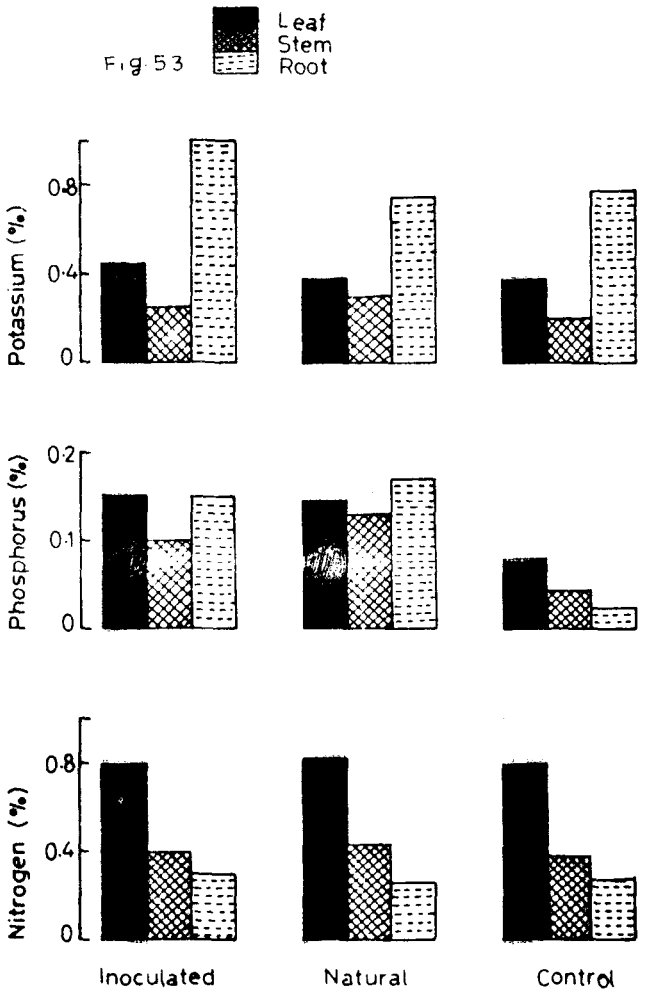

Leaf
Stem
Root
Potassium (%)
0·8
0·4
0
Phosphorus (%)
0·2
0·1
0
Nitrogen (%)
0·8
0·4
0
Inoculated
Natural
Control

Table: 6.1 The infection of vesicular-arbuscular mycorrhizae at different harvests.

Harvest periods	Inoculated			Natural.		
	Infection (%)	Vesicles (%)	Intensity	Infection (%)	Vesicles (%)	Intensity
2 months (I harvest)	43	20	+	82	18	+++
4 months (II harvest)	90	18	++	88	22	++++
6 months (III harvest)	71	33	+++	86	21	++++

sterilized and inoculated ones but the initial increase in the growth of the former over the latter, may be attributed to the earlier stimulating effect of the mycorrhizal infection which was very high since the beginning. However, a depression in growth, preceding heavier infection, in case of inoculated plants may also be accounted for this (Cooner, 1975). A reduction in the mycorrhizal infection in the inoculated plants at last harvest was not usual but the faster rate of growth of the roots than the rate of spread of infection may be the reason (Smith, 1982, discussion part). This was evident from the root dry weight production (Fig. 5.1) and also the root/shoot ratio (Fig. 5.2).

The high root/shoot ratio value of the control plants than both the mycorrhizal treated plants was due to the greater allocation of the resources towards the faster growth of the root than the shoot in the nutrient deficient condition in the control plants as a result of absence of mycorrhizal association (Chapin, 1980). The decrease in the root/shoot ratio in the control plants over the mycorrhizal counterparts at the last harvest was probably due to the strong establishment of the roots in the soil in the beginning and the faster allocation of the resources towards the shoot growth at latter stage.

The higher phosphate percentage present in the leaf, stem as well as root of the natural mycorrhizal plants was probably due to the greater mycorrhizal activity which was

evident from both the higher infection percentage and intensity of infection (Table 6.1). It may be assumed that the higher phosphate accumulation in the natural mycorrhizal plants, particularly in the root has some adverse effect on the normal growth of the plants, and due to this it could not compete with the growth of inoculated plants at advance stages of growth. The phosphate induced toxicity has also been reported by Mosse (1973b). Moreover, the population of the associated microorganisms in case of unsterilized natural soil was manifold high in comparison to the sterilized soil of the inoculated treatment and the probability of the presence of some parasitic organisms affecting the healthy growth of the root and the plant as a whole in turn cannot be ignored completely.

The potassium content in the inoculated plants, particularly in root was considerably higher than the control and the natural mycorrhizal plants and if the higher dry weight of the root and also the shoot tissues are taken into account, the concentration of the potassium in the mycorrhizal plants would be considerably higher than the non-mycorrhizal control. The dilution of the nutrient in the larger volume of plant tissues of the mycorrhizal plant has also been discussed by Menge et al., (1978c). The possibility of the uptake of other minerals, besides phosphate, by vesicular-arbuscular mycorrhizae has been indicated by Janos (1975). The potassium uptake in the plants through the mycorrhizal channel may be taken as spontaneous phenomenon. In the nutrient deficient soils the enhanced uptake of the potassium is facilitated by

VA-mycorrhizae in the field conditions (Mosse, 1973a). However, the increased potassium uptake of the mycorrhizal plants is probably the indirect effect of improved phosphate nutrition (Smith et al., 1981). The gradual increase in the growth of the inoculated plants, compared to the arrested growth of the natural mycorrhizal plants, may be attributed to the gradual development of the mycorrhizal infection and nutrient uptake, subsequently, which might have averted the accumulation of the nutrients to a toxic level, because of the continuous dilution of the nutrients in the larger volume of the plant tissues. It may be concluded that the inoculation with the VAM fungi in sterilized soil is more effective in promoting the growth of the plants than growing in natural unsterilized soil.

The effect of mycorrhizal inoculation (*Glomus tenuis*) in combination with *Trichoderma viride* Press. Gray on the growth of *Eupatorium adenophorum* Spreng.

Introduction

The microorganisms present in the rhizosphere of the plants are influenced by the root exudates and these microorganisms in turn, provide the available nutrients to the plants, releasing them from the unavailable sources, through their enzymatic activities. The mycorrhizal inoculation under sterilized soil condition, no doubt helps plant, in nutrient uptake and growth subsequently, but it also restrict the full utilization of the resources, which could have been exploited in a better way, through the activity of the intact microbial system in soil. According to Mosse (1975) "contaminating microorganisms associated with different endophytes can also have small but significant effects on the growth of the host". The present work was designed to study the effect of mycorrhizal fungus *Glomus tenuis* on the growth of *Eupatorium adenophorum* in presence of the saprophytic fungus *Trichoderma viride*.

Material and Methods.

G. tenuis was selected as the mycorrhizal inoculum for the present experiment because, it also formed the symbiotic relationship with the root of *E. adenophorum*, under natural condition (Chapter 1). Secondly its "fine mycelium" is highly

CHAPTER VII

efficient in phosphate uptake from the soil (Powell and Daniel, 1978). The reason behind the selection of *Trichoderma viride* was its constant presence in the rhizosphere of *E. adenophorum*. Further, the low pH has been reported to favour the activity of *Trichoderma* (Chet and Baker, 1980).

The soil used for the experiment was acidic with the soil properties as follows: pH 5.3, organic matter 2.6 (%), total nitrogen 0.19 (%) available phosphorus 4.2 (ppm) and exchangeable potassium 0.15 (mg/g) The soil was sterilized in autoclave and 1 kg of the sterilized soil was filled in the plastic pots of 1.5 kg capacity.

The seeds of *Eupatorium adenophorum* were surface sterilized by 0.1 % mercuric chloride (0.1 g dissolved in 100 ml, 75 % alcohol) and germinated at 30°C on water agar medium.

Preparation of mycorrhizal inoculum:- The strain of *Glomus tenuis* isolated and maintained on *Eupatorium adenophorum*, under glass house conditions was used as the mycorrhizal inoculum. The roots infected with *G. tenuis* were first washed thoroughly in tap water and then were cut into small pieces. In order to get rid of the contaminating microorganisms, they were surface sterilized with 0.1 % mercuric chloride, and washed in a series of sterilized distilled water. The procedure was standardized by earlier trials and it was ensured that no fungal species develop from the finally washed roots, in Czapek-Dox-agar medium.

Preparation of spore suspension:- Some spores of *T. viride* cultured on Czapek-Dox-Agar medium, were transferred to a conical flask containing 100 ml sterilized distilled water aseptically, and were stirred on a mechanical shaker for 15 minutes. The number of spores per ml. of suspension was measured according to Seeley Jr. and Vandemark (1970), as follows:

0.01 ml of the suspension was spread on 1 sq. cm. area marked in the middle of a slide. Then the marked area was observed under microscope and the spores present in 25 microscopic fields were counted.

The number of microscopic field present in the 1 sq. cm. area of slide was determined as below:

The area of microscopic field = πr^2

The number of microscopic field per sq. cm. = $1/\pi r^2 = X$

As 0.01 ml. of spore suspension was spread on 1 sq. cm. area, so, each microscopic field covered 0.01 or $\frac{1}{100} \times \frac{1}{X} = \frac{1}{Y}$ ml. of spore suspension.

Therefore, each spore in a field represented Y number per ml and hence, the mean number of spores (in a microscopic field) x Y, gave the number of spores present in 1 ml of suspension. Accordingly, 450, 900, 1500, 7500, 15000 and 30000 spores were mixed to the top sterilized soils of the pots separately. The pots used for control experiment were kept free from *Trichoderma* inoculation.

Further, 3 lots of 2 gs mycorrhizal inoculum were placed 2 cm. below the upper soil surface at 3 places in a pot and 7 days old seedlings were transplanted on them, ensuring

that the root of the seedlings got contact with the mycorrhizal inoculum. In this way, 3 seedlings per pot and 3 pots per preatment were maintained as replicates. The plants were watered with sterilized distilled water at every alternate day for 2 weeks and thrice a week afterwards. The experiment was conducted during May-Oct, 1981 and the only harvest was taken after 18 weeks.

The dry weight of the plant material, the percentage mycorrhizal infection and the shoot phosphate concentration were determined as described in chapter 4. Similarly the soil analysis for pH, organic matter, N, P and K was done as mentioned in chapter 1.

Results and discussion

The additions of Trichoderma viride in the soil favoured the growth of the plant with respect to root, shoot and total biomass production (Fig. 6.1). However, the increase in growth was not found to be significant statistically. The root/shoot ratio did not exhibit any regular pattern (Fig.6.1). The mycorrhizal infection was recorded higher (above 60 %) at every level of spore concentrations of T. viride and it showed significant positive relationship ($r = 0.791$, $P > 0.01$) with the phosphate concentration of shoot. This observation was contrary to Sanders (1975) who obtained negative relationship between phosphate concentration and the mycorrhizal infection of the plant. However, when the percentage infection

is considerably higher, even the little fluctuation in the intensity of infection may presumably result into changes in phosphate uptake of the plant. This is particularly important in case of G. tenuis which is highly efficient in phosphate uptake (Powell and Daniel, 1978).

The saprophytes in soil initiate the enzymatic degradation of the organic matter, releasing thereby the otherwise unavailable nutrients. These released nutrients (P in particular) is readily absorbed by the plants through the mycorrhizal channel. The synergistic effect of another saprophytic fungus Cylindrocarpon was also observed by Paget (1975). Manjunath et al., (1981) reported the beneficial effect of the combined treatment of a VAM fungus (Glomus fasciculatus), saprophytic fungus Aspergillus niger and a bacterium on the growth and nutrient uptake of onion plant but they also failed to obtain the significant increase in growth in case of single treatment of A. niger.

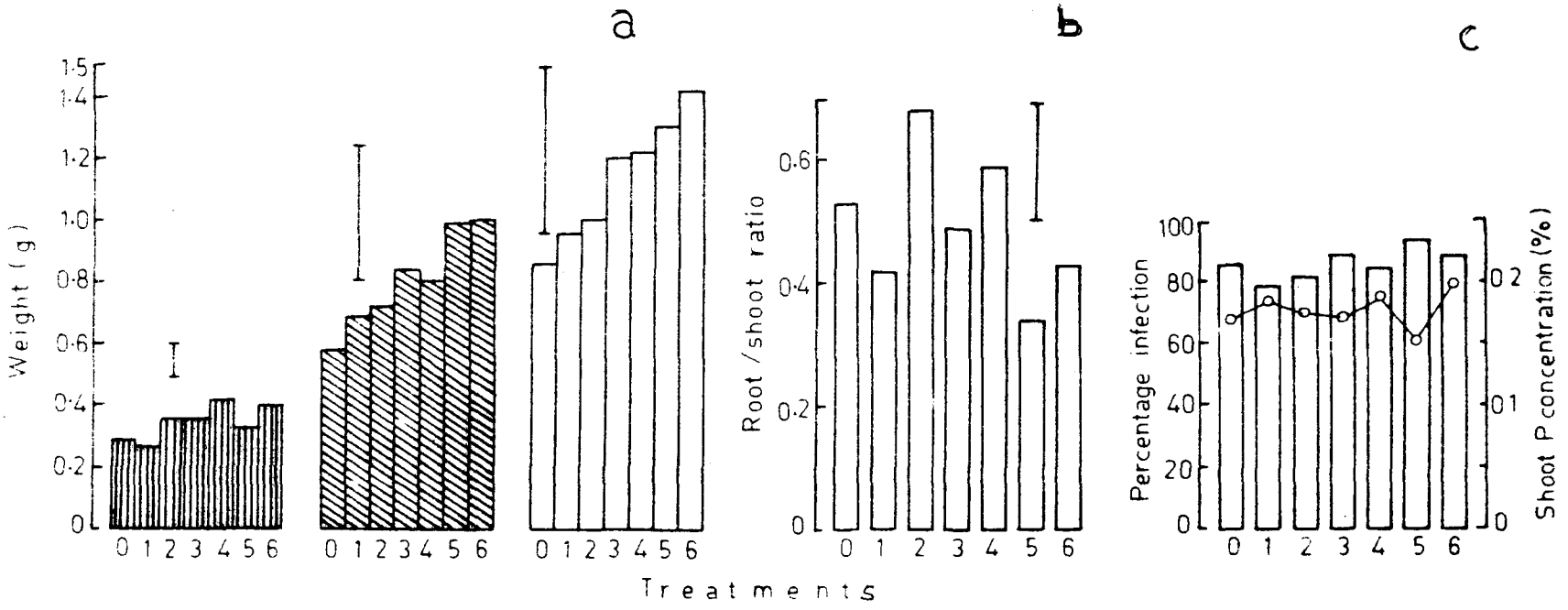
Although, the additions of T. viride did not increase the growth of E. adenophorum significantly but it indicated favourable influence towards the increasing growth and phosphate uptake. Therefore, it can be presumed that if the suitable microorganisms are screened and inoculated along with the mycorrhizal inoculum it might increase the yield.

Fig. 6.1 a The effect of the combined treatment of mycorrhizal endophyte *Glomus tenuis* and the saprophytic fungus *Trichoderma viride* on the growth of *E. adenophorum*. ▥ , root biomass; ▨ , shoot biomass; □ , total biomass.

b The root/shoot ratio under similar conditions.

c The relationship of percentage mycorrhizal infection and the percentage shoot phosphate concentration under similar conditions. Histograms, percentage mycorrhizal infection; O——O, shoot phosphate concentration. Concentrations of the spores of *Trichoderma viride*, 0, no spore; 1, 450 spores; 2, 900 spores; 3, 1500 spores; 4, 7500 spores; 5, 15000 spores; 6, 30000 spores.

Fig-6.1


a
b
c
Weight (g)
1·5
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
0 1 2 3 4 5 6
0 1 2 3 4 5 6
0 1 2 3 4 5 6
Root / shoot ratio
0·6
0·4
0·2
0
0 1 2 3 4 5 6
Percentage infection
100
80
60
40
20
0
Shoot P concentration (%)
0·2
0·1
0
0 1 2 3 4 5 6
Treatments

GENERAL DISCUSSION

The vesicular-arbuscular mycorrhiza is so prevalent in nature that "it is far easier to list the plant families in which they have not been found than to compile a list of plant families in which they are known to occur" (Gerdemann, 1975). The symbiotic relationship of host and the mycorrhizal fungi have developed probably from the time when the land flora came into existence (Nicolson, 1967) and this collateral evolution has achieved so much interdependence that their survival in separation is difficult to think (Harley, 1969). The spores produced by the VAM fungi is also universal and practically all soils, irrespective of the vegetation structure are in posession of *Endogone* spores (Nicolson, 1967; Gerdemann, 1968).

The external extensions of the mycelium beyond the reach of the roots, provide the large surface area to absorb the nutrients, which are transported back to the host. "Despite their relatively small biomass, the mycorrhizal fungi (mycobionts) are vital for uptake and accumulation of ions from soil and translocation to hosts because of their high metabolic rate and strategically diffuse distribution in the upper soil layers", Trappe and Fogel (1977).

The nutrient deficient soils are the sites of greater mycorrhizal activity. The function of the mycorrhiza has mainly been associated with the phosphate uptake in the phosphate deficient soils (Mosse, 1973a).

The soil of north-east India is phosphate deficient and favourable for greater mycorrhizal activity (Mosse, 1973a). Higher mycorrhizal infection was recorded (Fig. 1.1,1.2) in the three important weed species, viz., Eupatorium adenophorum, E. riparium and Osbeckia crinita, throughout the year (1979-80 and 1980-81). Winter season was found to have the maximum favourable effect on the mycorrhizal infection percentage (Fig. 1.1, 1.2). Similar was the observation of Sparling and Tinker (1978a). The annual plant species i.e. Osbeckia crinita showed a general increasing pattern of infection with the age of the plant, as was observed by Hayman (1970) in case of wheat in one crop-cycle. But, this pattern was not followed by the other two perennial species i.e. E. adenophorum and E. riparium. The high average annual rainfall and comparatively long and severe winter period (Fig. 1) appeared to be the controlling factors of the local environment and the same was reflected in the mycorrhizal behaviour. The rainfall, the maximum and the minimum temperature showed mostly negative relationship with the percentage infection and the Endogone spore population (sometimes significant at 1% level).

The strains of Glomus tenuis forming the distinguished "fine mycelium" in the root cortex of the host plants was found to be the most efficient endophyte of the winter months (Fig. 1.3). The observations of Crush (1973) and Haselwandter and Read (1980) is in agreement with this finding that the lower temperature favours the activity of G. tenuis.

The depth-wise study of the distribution of roots, the

mycorrhizal infection and the Endogone spore population indicated a general decrease, along the increasing depth in the vertical column of the soil (Fig. 2.3, 2.4). The trend was more or less similar to Sparling and Tinker (1978a), Sutton and Barron (1972) and Schwab and Reeves (1981).

A study in relation to the disturbances caused by the prevalent agriculture practice of the north-east India revealed some interesting and alarming informations. The shifting cultivation, which consists of the cutting of the existing vegetation including tree species and burning of the same after drying before the cultivation, has initiated a lot of imbalances in the proper functioning of the natural ecosystem. As the period of burning is immediately followed by the rainy season, when usually the crops are sown, the nutrient rich top soil is washed along with the running water. In this way, not only the nutrients, but the most valuable ingredients of the soil community, the Endogone spores are also removed, as they occur mostly in upper soil layers (15 cm. depth, Mosse, 1973a). The exceptionally higher population of Endogone spores at Site II when the quantity of the root and the mycorrhizal infection of root was measurably less in the rainy season (Fig. 2.1) is self explanatory that the spores would have been carried along with the eroded soil from the adjoining hills. This has resulted into the excessive deposition of the organic matter and other nutrients (N,P,K) at the valley lands (Site II), far more than the actual consumption, at one extreme and measurably poor nutrients concentration at another

extreme at hills i.e. Site I (Fig. 2.2, 2.5), not even sufficient to support the normal growth of plants. Under the circumstances the mycorrhizal fungi are supposed to play significant role in converting the unavailable sources of nutrients, particularly, the phosphate, to the available forms and supplying the same to the host plants. But, the experiment conducted to test the infective potential of the contrasting sites indicated that soils of the disturbed lands (Site I) have lost the infective power probably due to the lower mycorrhizal inoculum potential (Table 3.2). The eroded soils are poor in mycorrhizal infection has also been reported by Hall and Armstrong (1979). These observations also indicate a probable alarming condition in the near future if this sort of disturbances are allowed to continue. The hypothesis of Reeves et al., (1979) in this regard is important to mention: that (1) Disturbance of soil leads to reduction and possibly elimination of propagules of mycorrhizal fungi (because host plants are reduced in numbers); (2) Reduced numbers of propagules leads to a lower potential for infection of new host plants; (3) Non-mycorrhizal species become established because normally mycorrhizal plants die in the seedling stage (for lack of mycorrhizal fungi); (4) Success of non-mycorrhizal species further reduces the propagules of mycorrhizal fungi since the fungi are obligate symbionts; (5) Total elimination of mycorrhizal fungi obviates competition by mycorrhizal higher plants; (6) Succession is slowed because of the lack of potential mycorrhizal fungi (these fungi may be slow invaders);

and (7) The harsher the site the greater the potential for elimination of mycorrhizal propagules. The process might be irreversible because the chlamydospores or azygospores are larger in size and poorly adapted to dissemination (Nicolson, 1975).

The glass-house experiments confirmed the observations of others (Sanders, 1975; Abbott and Robson, 1977b; Menge et al., 1978a; and Jasper et al., 1979) that the additions of phosphate sources, in available or unavailable forms inhibited the mycorrhizal infection (Fig. 3.1a). The effect of mycorrhizal fungi G. tenuis in presence and absence of phosphate (Na_2HPO_4), on the growth of Eupatorium adenophorum showed that after certain level of pohsphate (0.4 g/3 Kg soil in this case) the growth of the non-mycorrhizal plants was at par with the mycorrhizal plants (Table 4.1).

The comparative study of the two methods of assessing the mycorrhizal infection viz., the chitin digestion method and the root slide method, indicated that the former was superior to the latter, generally, but in case of lowest infection (Fig. 3.1a, at 5 g, P/pot) the sensitivity of the chitin digestion method was found to be doubtful. Similar doubts have been expressed by Tatareau and Touze (1975), Hepper, (1977), Sakurai, et al., (1977) and Sharma et al., (1977) on the ground that the age of the fungal mycelium, the structural pattern of the mycorrhizal fungi within the root cortex or the environmental changes, may alter the composition of fungal chitin. Whipps and

Lewis (1980), suggested to follow some other histological observations simultaneously with the Chitin digestion method in order to get more accuracy in result. Similarly, Hepper (1977) opined to use the Chitin assay method in combination with the root slide method.

The different strains of mycorrhizal fungi differ in their effect on the growth of the plants. Two strains viz., Glomus fasciculatus and G. mosseae native to U.S.A. and U.K. respectively, when introduced to soil (in pot experiment) were found superior to the local endophyte G. tenuis in promoting the growth and nutrient uptake in case of E. riparium. The variable effect of different mycorrhizal strains on the growth of the various host plants has been observed in a number of studies (Mosse, 1972b; Powell, 1975b, 1979b; Hall, 1976). Similarly, it has been reported that the introduced endophytes are superior to the indigenous ones in promoting the growth and nutrient uptake (Mosse and Hayman, 1971; Mosse, 1975, 1977). Among the introduced endophytes, G. fasciculatus was found to be highly efficient in favouring the enhanced growth of Eupatorium riparium (Fig. 4.1). The efficient functioning of G. fasciculatus in acid soils has been reported by Gilmore (1968), Hall and Fish (1979), Mosse (1972a) and Powell (1976). Mosse (1972a) also indicated the inefficiency of G. mosseae in acid soil. Though G. tenuis showed the poorest growth performance among all the mycorrhizal strains, in combination with G. mosseae it enhanced the shoot growth, however, in combination with G. fasciculatus it failed to do so (Fig. 4.1).

G. tenuis had also contrasting effect on the colonization of the root cortex in combined treatments. At one hand, the development of G. tenuis was checked by G. fasciculatus, while on the other hand, the same G. tenuis checked the spread of G. mosseae (Table 5.1).

In general, the nitrogen, phosphorus and potassium concentration in the root of the combined treatment of G. fasciculatus + G. tenuis and G. mosseae + G. tenuis was higher than other mycorrhizal treatments. The efficiency of G. tenuis in greater uptake of phosphate has been reported by Powell and Daniel (1978). The other nutrients are also absorbed by the mycorrhizal fungi passively along with the increased uptake of phosphate (Janos, 1975; Smith et al., 1981). The interesting observation in case of G. mosseae was that it helped in potassium uptake more than other mycorrhizal fungi (Fig. 4.3). The possible role of mycorrhizal fungi in potassium uptake has been indicated by Mosse (1973a) and Powell (1975c).

The sterilization of soil in order to remove the existing mycorrhizal propagules, also removes the associated microorganisms. The experiment conducted with Osbeckia crinita under sterilized and unsterilized soil conditions indicated that the inoculation of VAM fungi in sterilized soil is comparatively better in promoting the growth of the plants than growing in unsterilized natural soils (Fig. 5.1). Although this observation was contrary to the expectation, however, it can be explained in two ways; firstly, the strong competition

faced by the VAM strains due to associated microorganisms and secondly, the probable presence of some parasitic microorganisms in the unsterilized natural soils.

But the other experiment with Eupatorium adenophorum indicated a different and expected result. The inoculation of Trichoderma viride, (a common saprophytic fungus frequently observed in the rhizosphere of E. adenophorum) along with the mycorrhizal fungi G. tenuis had the favourable effect on the growth of the plant compared to the Trichoderma free mycorrhizal inoculation treatment (Fig. 6.1). The result was although not significant statistically but the trend was favourable and encouraging and therefore it can be concluded that if a proper screening of the soil microorganisms is done and the efficient strains of the same is added with the mycorrhizal inoculum it may increase the yield.

SUMMARY

The agriculture system prevalent in the north-east India is the shifting cultivation type which includes the cutting of the existing vegetation and burning of the same after drying. The time period of slash and burn is as such that the burning is immediately followed by rainy season, when the cultivation is done usually. At this stage, a number of weed species invades the burnt sites. The Eupatorium adenophorum, Spreng, E. riparium, Regel, (Asteraceae) and Osbeckia crinita Wall. ex. D.. Don (Melastomataceae) are among the early colonizers which occupy the vast area of the cultivated land and forest.

The vesicular-arbuscular mycorrhizal studies of these weed species revealed that they are highly mycotrophic as considerably higher mycorrhizal infection was recorded throughout the year (1979-80 and 1980-81). Based on morphological characters, three types of VA-mycorrhizal fungi were distinguished which dominated within the root cortex, under the influence of seasonal variation. The strain of Glomus tenuis was favoured by the low temperature of winter months, while the Gigaspora species appeared to be active during summer months. A third type of infection observed was probably due to some strain of Glomus species.

Similarly, the Endogone spore population was also found to be higher in the root region of these weed species. Among the spores the members of Gigaspora, Sclerocystis and

Glomus species were observed but the majority of spores belonged to Glomus species.

The studies of the fungal flora of the rhizosphere, showed that Osbeckia crinita attracted the least number of fungal species. The highest number of fungal flora was recorded in case of Eupatorium adenophorum followed by E. riparium. The most common fungi, isolated frequently were the yeasts, Trichoderma viride, Penicillium sp., Phoma humicola and Sterile mycelium.

In a depth wise (upto 30 cm) study it was found that the distribution of roots, the mycorrhizal infection of the roots and the Endogone spore population, all showed a decreasing pattern along the depths of soils. The pot experiment conducted with the soils of different depths and contrasting sites (one highly disturbed hilly land and another relatively less disturbed valley land) indicated that the highly disturbed sites have lost the mycorrhizal infective power due to lower inoculum potential of the mycorrhizal spores (Endogone).

The highly mycotrophic nature of the Eupatorium species were further confirmed by the pot experiments in glass house. The additions of soluble phosphate (Na_2HPO_4) inhibited the mycorrhizal infection and at the highest phosphate level (5 g/3 Kg soil) the infection percentage was reduced to 16 % only. The growth of non-mycorrhizal plants was similar to the mycorrhizal plants at the higher phosphate levels (after 0.4 g/3 Kg soil). The growth of the mycorrhizal plants however,

was found to be far better than the non-mycorrhizal plants when grown without phosphate.

The introduced endophytes, G. fasciculatus and G. mosseae, native of U.S.A. and U.K. respectively, when inoculated to Eupatorium riparium, in pot experiments, enhanced the growth and nutrient uptake, far better than the local endophyte, G. tenuis. However, the combination of G. tenuis and G. mosseae (1:1) induced greater shoot growth while the combination of G. tenuis and G. fasciculatus retarded the same.

The microorganisms of the soil play significant role in the nutrient cycling of the ecosystem. The mycorrhizal activity under natural unsterilized soil condition (with intact associated microorganisms) and sterilized condition (without associated microorganisms) revealed that the inoculation of mycorrhizal fungi in sterilized soil has greater benificial effect on the growth of the plants (Osbeckia crinita in this case) because of the competition free environment. However, another experiment done with E. adenophorum indicated that the inoculation of the saprophytic fungus Trichoderma viride, in association with the mycorrhizal fungus G. tenuis had synergistic effect on the growth and phosphate uptake. The contrasting result of the two experiments suggested the middle path i.e. the screening of the rhizosphere microorganisms and inoculation of only the efficient ones, in association with the efficient mycorrhizal fungi would have more beneficial effect on the growth and nutrient uptake of plant than the inoculation of mycorrhizal fungi singly.

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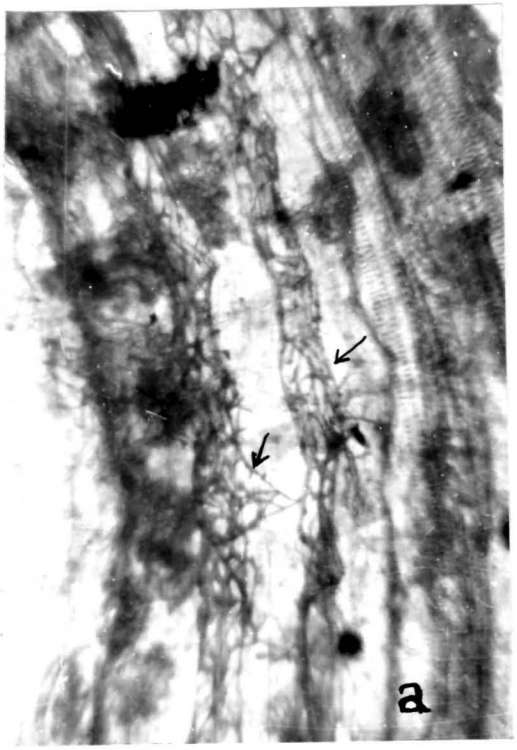
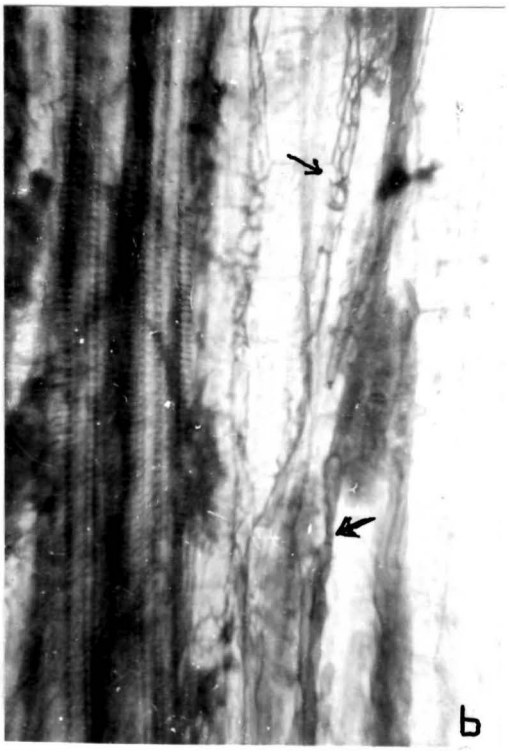
Plate 1 Effect of different doses of phosphate on the growth of *E. adenophorum*. 1, 0 g; 2, 0.1 g; 3, 0.2 g; 4, 0.4 g; 5, 0.6 g; 6, 0.8 g; 7, 1.0 g; 8, 3.0 g; 9, 5.0 g. The sequence is non-mycorrhizal and mycorrhizal in each treatments.

PLATE - 1

Plate 2 The colonization of mycorrhizal endophyte in root. a, the heavy colonization; b, moderate colonization; c, very sparce colonization by mycorrhizal fungi at 0.1, 0.8 and 5.0 g phosphate levels in soil respectively.





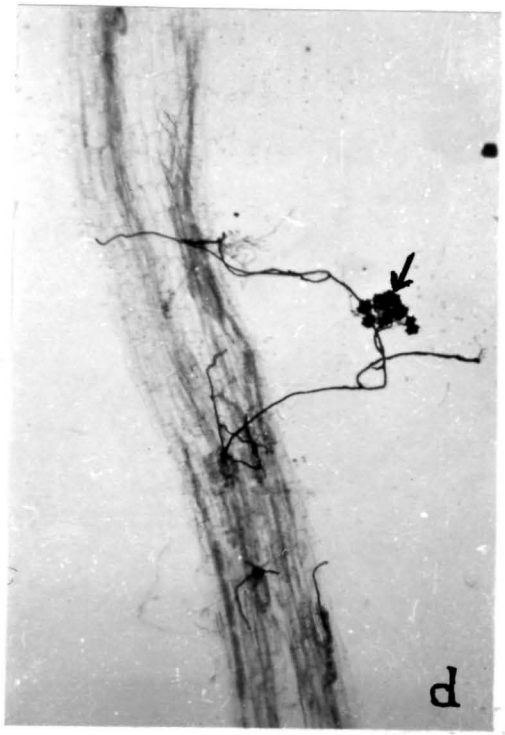
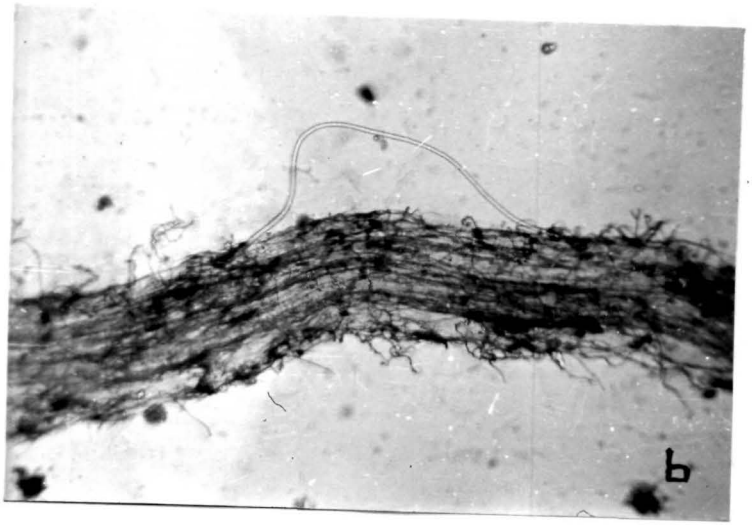


PLATE - 3

Plate 3 Occurrence of different strains of mycorrhizal fungi under the influence of seasonal changes in the plants growing in nature. a, fine endophyte in *E. riparium*; b, fine and coarse endophytes together in the root of *E. adenophorum*; c, vesicles structure in *E. adenophorum*; d, external vesicles, the characteristic of *Glomospore* sp., in *E. adenophorum*.



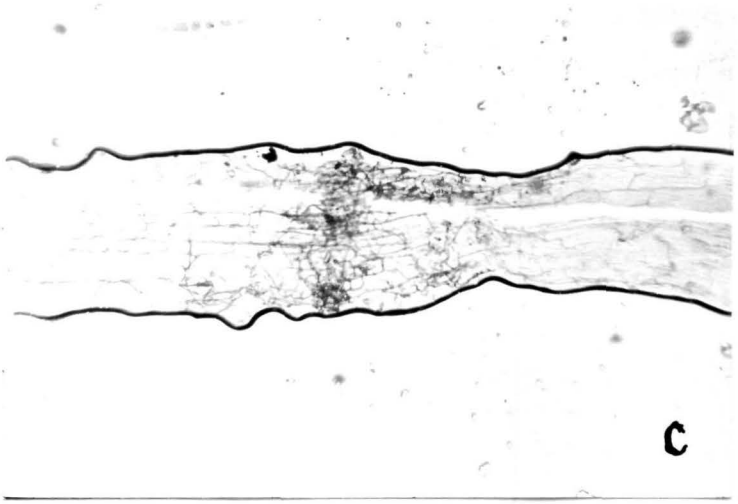


PLATE - 2

Plate 4 a, Sporocarp of *Glomus* sp. attached with the root of Usbeckia crinite; b, extramatrical spore production by *G. riparium*, under natural conditions.

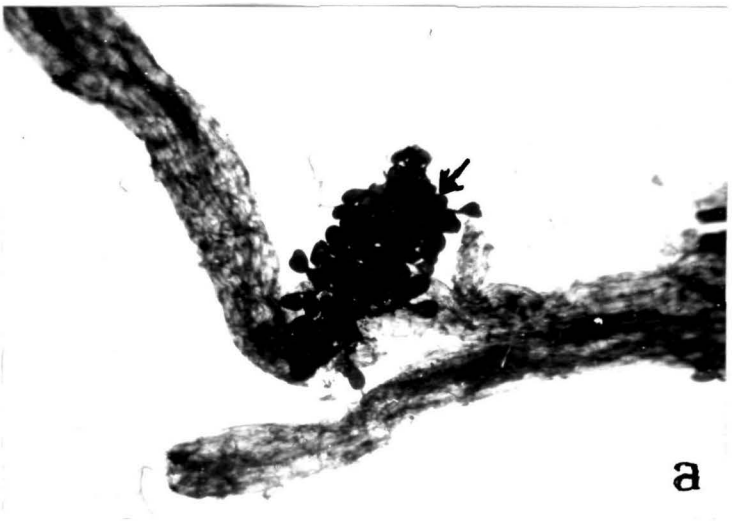
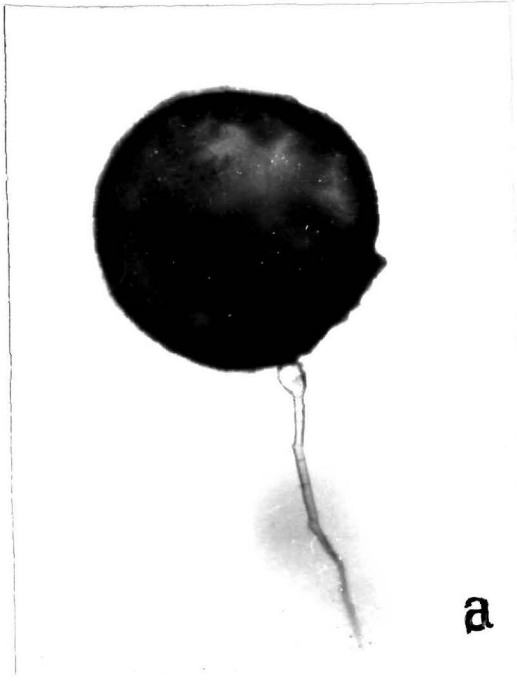


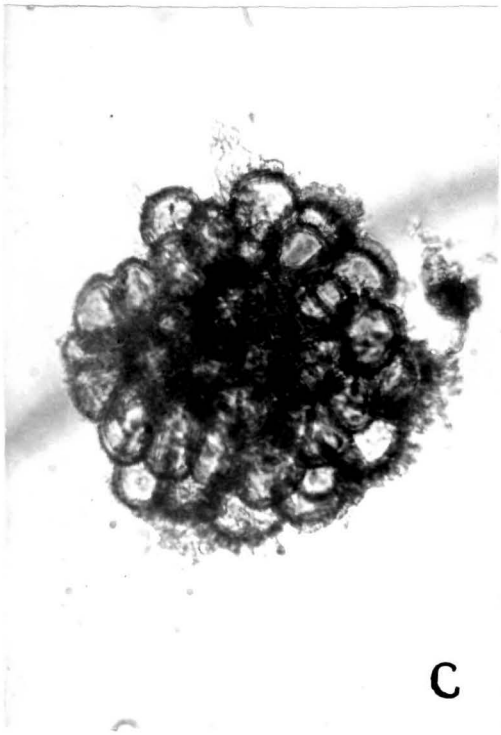
PLATE - 4

Plate 5 The different types of spores isolated from the soil. a, *Gigaspora* sp. 1; b, *Gigaspora* sp. 2; c, *Sclerocystis* sp.; d, *Glomus* sp.



a

b



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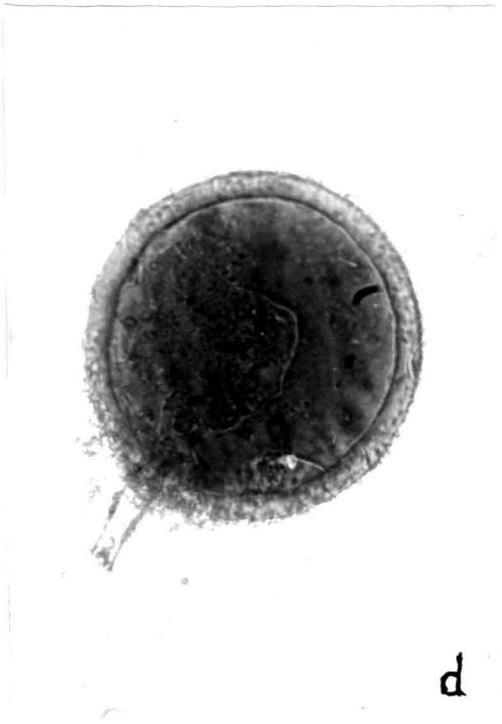
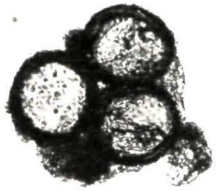


d

PLATE - 5

Plate 6 The different types of *Glomus* sp. isolated from the soil. a, sporocarp of *Glomus* sp. 1; b, sporocarp of *Glomus* sp. 2; c, spore of *Glomus* sp.



a

b

c

PLATE - 6